

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

November 4, 2018 | 8:00 AM – 12:00 PM

Session Chair: Kyathanahalli Janardhan, BVSc, MVSc, PhD, DACVP, Integrated Laboratory Systems, Research Triangle Park, NC

November 4, 2018

9:00 AM – 9:30 AM

### **ACETALDEHYDE POTENTIATES THE TUMOROGENESIS OF N'-NITROSONORNICOTINE IN RATS**

Flaviu Tabaran, Ingrid Cornax, Stephen Hecht, Silvia Balbo, Lisa Peterson, Michael O'Sullivan

**Background:** *N'*-Nitrosonornicotine (NNN) and acetaldehyde are major constituents of tobacco smoke. NNN acts as a potent oral carcinogen by forming pyridyloxobutyl DNA adducts following metabolic activation. Acetaldehyde is a strong electrophile and acts as a direct genotoxicant, inducing DNA adducts and cross-links and inhibiting DNA repair. Since tobacco smoke is a complex mixture of toxic and carcinogenic chemicals, studying the interaction of specific tobacco chemicals increases the relevance of toxicologic studies to understanding health risks associated with smoking tobacco.

**Objective:** To investigate whether acetaldehyde potentiates the carcinogenic effect of NNN with respect to tumorigenesis.

**Methods:** Nine groups of male F344 rats (n=15) were exposed in the drinking water to 0, 4 or 8 ppm NNN for up to 100 weeks in the presence or absence of 3000 ppm acetaldehyde. Oral and esophageal lesions in animals surviving beyond 69 weeks were classified as atypical epithelial hyperplasia (AEH) or squamous papilloma (SP).

**Results:** NNN dose-dependently significantly increased the number of animals with AEH (n=6 of 10 vs n=3 of 12 animals) and the number of SP (n=9 vs n=2), typically involving esophagus, for the 8 and 4 ppm groups, respectively. Acetaldehyde alone was not tumorigenic, but significantly increased the number of NNN-exposed rats exhibiting AEH (n=13 of 13 and n=7 of 12 animals, 8 vs 4 ppm groups respectively), and significantly increased the number of SP in the 8 ppm group (n=20) although not the 4 ppm group (n=2).

**Conclusions:** Acetaldehyde potentiates the oncogenesis of *N'*-nitrosonornicotine in F344 rats.

November 4, 2018

9:30 AM – 9:45 AM

### **THE SINOATRIAL NODE REGION OF THE BEAGLE DOG: SECTIONING PROCEDURE, HISTOLOGY, AND BACKGROUND PATHOLOGY**

Rebecca Terry, Rachel Pittaway, Ian Taylor, Peter Clements

**Background:** Over the last six years we have used a standardised sectioning procedure to examine the sinoatrial (SA) node region of the right atrium in beagle dogs on toxicology studies. Mixed perivascular inflammatory cell infiltrates surrounding the SA nodal arteries, sometimes accompanied by a range of mural pathology, have been seen in dogs dosed with several unrelated compounds.

**Objective:** Retrospective study to determine the background incidence of inflammatory findings in the SA node region in control beagle dogs from the last 6 years.

**Methods:** Sections of the SA node region from 181 control beagles (strain HsdRCC:DOBE) from oral, inhalation and intravenous toxicology studies were examined (90 females and 91 males, from 6 to 18 months old). Dogs were only included if the SA nodal arteries and nodal tissue were captured in at least one section. Sectioning procedure, representative images of the normal anatomy, variations in histological appearance of normal vessels and lesions were characterized.

**Results:** Minimal to mild perivascular inflammatory cell infiltrates were seen in 1 female and 2 males with intimal proliferation, discontinuity of the elastic lamina and medial hypertrophy in mild lesions. Fibrinoid necrosis was not seen. Other findings in the SA node region included: segmental medial hypertrophy of a SA nodal vessel (without inflammatory infiltrates) in 1 male, minimal focal mononuclear cell infiltrates within the myocardium of 2 males, subepicardial mononuclear cell infiltrates in 2 females and mesothelial hyperplasia in 1 male.

**Conclusion:** This novel background data will be a useful reference for toxicology studies.

November 4, 2018

9:45 AM – 10:00 AM

#### **INVESTIGATION OF SILVER DEPOSITION AND INFLAMMATORY LESION IN SELECTED ORGANS OF STRANDED CETACEANS**

Wen-Ta Li, Bang-Yeh Liou, Wei-Cheng Yang, Meng-Hsien Chen, Hui-Wen Chang, Victor Fei Pang, Chian-Ren Jeng

**Background:** Silver nanoparticles (AgNPs) have been extensively used in commercial products and released into the environment. Therefore, AgNPs have been considered a potential source of Ag contamination and raise the public concern about the environmental toxicity. Cetaceans as apex predators of the ocean have been shown to have a relatively high level of Ag in their bodies. Although the immunotoxicity of AgNPs have been demonstrated in cetaceans, the correlation between Ag deposition and the lesion in cetaceans is poorly understood.

**Materials and Methods:** Tissue samples, including central nervous system, lung, heart, liver, kidney, gastrointestinal tract, were collected from 54 stranded cetaceans. The correlation between the lesion variables and Ag concentrations in the liver of each individual specimen were investigated. The lesion score was obtained as: absence

[score: 0] or presence [score: 1] of inflammatory lesions in each organ, and total lesion scores were the accumulation from six organs of each individual.

**Results:** Total lesion scores (TLSs) and the periportal hepatitis were positively correlated with Ag concentrations (Spearman's rho;  $r = 0.2731$ ;  $p = 0.0453$  and  $r = 0.3752$ ;  $p = 0.0052$ ). Although only an increased trend of Ag concentrations was noted with increased TLSs, Ag concentrations were significantly higher in stranded cetaceans with periportal hepatitis (Mann-Whitney U test;  $p = 0.0056$ ).

**Conclusion:** This study demonstrated the possible negative health effects of Ag in cetaceans, suggesting that 1) Ag is direct-acting toxicants and inducing damage to the periportal hepatocytes and inflammatory response, and/or 2) the periportal hepatitis is induced by an autoimmune response due to the immunotoxicity of Ag.

November 4, 2018

11:30 AM – 12:00 PM

### **AN OUTBREAK OF HYPERVITAMINOSIS D IN RABBITS DUE TO A FEED MILL MIXING ERROR**

Jacqueline Kurz, Thomas Baldwin, E Jane Kelly, Gordon Hullinger, Jeffery Hall

Nine rabbits from five separate submitters were diagnosed with vitamin D toxicosis based on a combination of gross and microscopic necropsy findings, elevated hepatic vitamin D, elevated serum calcium and phosphorus, and/or elevated renal calcium and phosphorus. Excess vitamin D in a feed sample was confirmed by liquid chromatography tandem mass spectrometry. Initial clinical presentation included weakness, weight loss, failure to breed, and increased mortality among neonatal to adult rabbits. The major pathologic finding in all rabbits was soft tissue mineralization of the renal cortices, vascular walls, tracheal walls, bronchial and bronchiolar walls, and various other tissues. Toxicosis resulted from severe dietary over-supplementation of vitamin D nearly thirteen times that reported to be toxic in rabbits. Toxicosis occurred due to a feed mill mixing error determined to have affected all nine rabbits, and estimated to have affected several thousand commercially- and privately-owned rabbits in multiple states. A delay of several months from the time of initial clinical presentation to submission of samples for diagnostic investigation resulted in severe and sustained losses in multiple rabbitries. These cases demonstrate the importance of prompt diagnostic investigation when non-specific clinical signs are observed among livestock populations.

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

November 6, 2018 | 1:30 PM – 5:00 PM

Session Chair: Kyathanahalli Janardhan, BVSc, MVSc, PhD, DACVP, Integrated Laboratory Systems, Research Triangle Park, NC

November 6, 2018

2:30 PM – 3:00 PM

### **THE CONTRIBUTION OF CORTICAL INTERNEURONS TO CEREBRAL BLOOD FLOW**

Catherine Ruff, Jay Couey, Alberto Vazquez, Sarah Ross

**Background:** The brain lacks energy reserves and relies on blood supply for energy substrates. In the cortex, the interplay between neurons, astrocytes and the vasculature ensure that adequate blood is supplied to brain regions with increased neural activity. Although cerebral blood flow (CBF) is critical to normal brain function and is compromised in many neuropathologies, the underlying neural basis remains unclear.

**Objective:** To elucidate the basic neural circuitry underlying activity-induced changes in cerebral blood flow, focusing on a subset of cortical inhibitory interneurons.

**Methods:** Here, we report the generation of a neurokinin 1 receptor (NK1R)-CreER knock-in mouse that selectively labels a subset of cortical inhibitory neurons that we suspect mediate CBF. Using this genetic tool, with a combination of immunohistochemistry, 2-photon microscopy, 3D whole-tissue imaging (CLARITY), electrophysiology, optogenetic techniques and in vivo laser Doppler flowmetry, we examined the connectivity and function of these cortical interneurons.

**Results:** We found that this population of interneurons possess long-range projections that closely track the microvasculature, co-express neuronal nitric oxide synthase (nNOS) and receive local excitatory input from pyramidal neurons (n = 7). Finally, in the awake animal, direct activation of these neurons via optogenetic stimulation leads to an increase in local CBF (n = 4).

**Conclusions:** These findings suggest that NK1R/nNOS-expressing cortical inhibitory interneurons act as local integrators of neural activity to mediate changes in CBF. This insight into the neural basis of CBF is fundamental to understanding the pathogenesis of cerebrovascular diseases and for the development of therapeutics targeting cerebral perfusion.

November 6, 2018

4:30 PM – 4:45 PM

### **STRAIN SUSCEPTIBILITY DIFFERENCES: EVALUATING MORIBUND STATUS, HISTOPATHOLOGY, AND SERUM BIOMARKERS OF CARDIAC INJURY IN THREE MOUSE STRAINS**

Eui Jae Sung, Susan Borghoff, Abraham Nyska, June Dunnick, Gregory Travlos

**Background:** Identification of cardiotoxic environmental hazard utilizing mouse models is important but challenging since the susceptibility to toxicants may differ depending on the strain used.

**Objectives:** The aim of this study was to evaluate differential cardiotoxicity in three mouse strains (B6C3F1, C3H/HeJ and C57BL/6J) with two different cardiotoxins (bis(2-chloroethoxy)methane (CEM) and ephedrine/cafeine) administration by moribundity, histopathology and serum biomarkers.

**Methods:** Mice (15 mice/strain/dose/chemical) were administered CEM at 0, 50, 100, 150, 200 or 300 mg/kg or mixtures of ephedrine/cafeine at 0/0, 12.5/30, 25/30, 50/30, 100/30 or 200/30 mg/kg, once a day, for three days. At necropsy, serum troponins I and T (TnI, TnT) and myosin light chain 3 (Myl3) were measured and heart histopathology was performed. Animals demonstrating clinical signs of toxicity, had blood collected and were sacrificed at the time of observation and counted as moribund.

**Results:** Mice administered CEM, but not ephedrine/cafeine, demonstrated increased moribundity and histopathological lesions. Mice administered a single CEM dose of 300 mg/kg caused mild myocardial vacuolation in 60%, 40% and 80% in B6C3F1, C3H/HeJ and C57BL/6J, respectively. Further, in all three strains, biomarker concentrations from moribund were much higher than those of non-moribund mice. Serum TnI and Myl3 concentration showed a positive trend with administration to both CEM and ephedrine/cafeine.

**Conclusion:** The C3H/HeJ strain was more resistant to CEM-related decreases in survival and cardiotoxic lesions compared to C57BL/6J and B6C3F1 mice. Furthermore, serum TnI and Myl3 was useful to determine cardiotoxicity, particularly when histopathological lesions were not present.

November 6, 2018  
4:45 PM – 5:00 PM

## **RETROSPECTIVE ANALYSIS OF FUNGAL RHINITIS IN SPRAGUE-DAWLEY RATS: SEX DIFFERENCES IN INFECTION FREQUENCY AND LESION LOCATION IN NASAL CAVITY**

Karen Carlton, Jennifer Lamoureux, Charissa Dean, Keith Nelson

**Background:** Fungal rhinitis is observed as a background finding in chronic toxicology studies in rodents and may contribute to premature deaths, thereby confounding interpretation of toxicologic findings.

**Objective:** We retrospectively evaluated data from thirty-two Sprague-Dawley rat 2-year carcinogenicity studies, including 25,781 animals from the same test facility, for findings of fungus and inflammation within the four H&E-stained sections of the nasal cavity.

**Methods:** Affected animals (175 total) from a subset of seven recent studies were re-examined, and the presence and/or severity of exudate, fungus, ulceration, invasion, and other specific findings, including the most affected location were recorded.

**Results:** Fungus uniformly had septate, parallel walls, and dichotomous branching, with occasional fruiting bodies, consistent with *Aspergillus* sp. The overall incidence was similar between treated and untreated animals, with males more frequently affected than females (3% versus 0.3%) and males more likely to have fungal rhinitis identified as the cause of morbidity/early death (31.6% versus 4.3%). Male rats were generally affected in the rostral two sections of the nasal cavity (90.8% of cases), and females within the caudal two sections (60.9% of cases). Usually fungus was identified in one half of the nasal cavity, accompanied by predominately neutrophilic inflammation of varying severity, with embedded plant or hair in approximately half of cases.

**Conclusions:** While the overall incidence is low, the incidence varied between studies, and fungal rhinitis can be a significant cause of morbidity in aged rats. The consistently observed wide variance in incidence and location between males and females is not readily explained and warrants further investigation.

## Industrial and Toxicologic Pathology Focused Scientific Poster Session

### T-01: BACKGROUND TESTICULAR LESIONS IN MALE NOD.CG-PRKDC-SCID-IL2RG-TM1WJL/SZJ (NSG) MICE IN PRECLINICAL TOXICOLOGIC STUDIES

Jimmy Tran, Michelle Elliott, Kathleen Szabo, James Raymond

**Background:** NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) mice are an established research model but their use in preclinical toxicologic studies remains uncommon. Published data on the type and incidence of background lesions in NSG mice from preclinical toxicologic studies are sorely lacking.

**Objective:** To present the background testicular findings in male NSG mice to aid interpretation of findings in preclinical toxicologic studies.

**Methods:** Analyzed were data from the male control group of 3 studies ranging from 44 – 183 days in duration (n = 57). NSG mice were sham treated with intravenous tail vein injections. Histopathology for each study was conducted by an independent pathologist.

**Results:** Findings in the testes of 57 male control NSG mice, from most to least common, were: seminiferous tubular degeneration/atrophy (n = 21), seminiferous tubular necrosis (n = 13), and interstitial (Leydig) cell hyperplasia (n = 2). Findings were both unilateral and bilateral. Seminiferous tubular degeneration/atrophy was of minimal to mild severity in most animals (n = 17/21). Seminiferous tubular necrosis was a novel background lesion characterized by coagulative necrosis with minimal inflammatory infiltrates. Seminiferous tubular necrosis was only seen in animals in studies over 88 days duration. Some affected animals showed systemic inflammatory lesions suggestive of opportunistic infection, which may have resulted in thrombosis and ischemic necrosis.

**Conclusions:** Seminiferous tubular necrosis is a novel background finding that can occur at high incidences in NSG mice used in preclinical toxicologic studies over 88 days duration. Seminiferous tubular degeneration/atrophy, as seen in other rodent models, was also noted.

### T-02: COMPARISON BETWEEN ADJUVANT-ASSOCIATED HISTOLOGICAL BACKGROUND FINDINGS AT INTRAMUSCULAR INJECTION SITES IN RABBITS

Elizabeth Goldsmith, Keith Nelson

**Background:** Intramuscular injection is commonly used for vaccine administration, with adjuvants often co-administered to stimulate the local immune response. In safety testing, the accurate recognition and quantification of compound-related findings and differentiation from adjuvant-associated findings at the injection site is important for evaluation of toxicity.

**Objective:** The purpose of this study was to review and retrospectively analyze 10 years of background histological findings seen at our facility with multiple

intramuscularly-injected adjuvants in control rabbits, a common model for vaccine safety studies.

**Methods:** Histologic data were tabulated for 381 rabbits, totaling 1215 injection sites from 15 intramuscular injection studies. Comparisons were made across a range of excipients, including saline and dextrose, and adjuvants, such as GLA-SE, PHAD, aluminum hydroxide (Alum™), and DPPC.

**Results:** The most common findings were chronic inflammation within muscle and subcutis and myofiber degeneration in muscle, occurring in almost all adjuvant or excipients examined. There was a higher prevalence (> 40%) of chronic intramuscular inflammation with GLA-SE and Alum™. Intramuscular foamy macrophages were seen with Alum™ (26.7%), GLA-SE (3.7%), and PHAD (1.7%). Alum™-dosed injection sites also had foreign material present.

**Conclusions:** Consideration of excipient- or adjuvant-related findings as well as those induced by the injection procedure will aid in evaluation of possible test article-related findings and should be a key component of any intramuscular injection study or evaluation of injection site pathology.

### **T-03: METHODS OPTIMIZATION FOR ROUTINE SCIATIC NERVE PROCESSING**

Jessica Fortin, Elizabeth Chlipala, Daniel Shaw, Brad Bolon

**Background:** Histopathological evaluation of the peripheral nervous system (PNS) is necessary to register chemical and pharmaceutical products. Recent “best practice” recommendations for PNS sampling and processing provide good but incomplete guidance regarding optimal methods for sciatic nerve evaluation.

**Objective:** This study explores fixation and processing methods to improve nerve preservation.

**Methods:** Sciatic nerves from adult rats and young pigs (n=4-6) collected at “0” (<15 min for rat and ~60 min for pig), 3, 6, 12, and 24 h of death were immersed in commercial neutral buffered 10% formalin containing 1.2% methanol (NBF) or methanol-free 4% formaldehyde (MFF) at room temperature (RT) for 24 h (rats) and 48 h (pigs). Specimens were processed into paraffin, and ~5-µm-thick sections were flattened on water baths set at 35°C, 40°C, or 44°C before mounting and H&E staining.

**Results:** For both small-diameter (rat, ~1 mm) and large-diameter (pig, > 5 mm) nerves, tissue and cytoarchitectural integrity was preserved if fixation occurred within 3 h by immersion in either NBF or MFF. At later times, myelin analysis was complicated by artifactual “bubbling” (appearing as an irregular mesh of clear vacuoles). Structural preservation of nerve fibers was acceptable using NBF but was better for MFF. Use of a water bath at 35°C reduced processing-related section dissolution (i.e., nerve fascicle separation) better than warmer temperatures.

**Conclusions:** Nerve tissue and cellular architecture is optimal when samples held at RT are fixed within 3 h of death. Nerves retain integrity best if sections are floated on cool water baths. These data provide further information to optimize PNS processing.

#### **T-04: THE CONTRIBUTION OF CORTICAL INTERNEURONS TO CEREBRAL BLOOD FLOW**

Catherine Ruff, Jay Couey, Alberto Vazquez, Sarah Ross

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**Objective:** To elucidate the basic neural circuitry underlying activity-induced changes in cerebral blood flow, focusing on a subset of cortical inhibitory interneurons.

**Methods:** Here, we report the generation of a neurokinin 1 receptor (NK1R)-CreER knock-in mouse that selectively labels a subset of cortical inhibitory neurons that we suspect mediate CBF. Using this genetic tool, with a combination of immunohistochemistry, 2-photon microscopy, 3D whole-tissue imaging (CLARITY), electrophysiology, optogenetic techniques and in vivo laser Doppler flowmetry, we examined the connectivity and function of these cortical interneurons.

**Results:** We found that this population of interneurons possess long-range projections that closely track the microvasculature, co-express neuronal nitric oxide synthase (nNOS) and receive local excitatory input from pyramidal neurons (n = 7). Finally, in the awake animal, direct activation of these neurons via optogenetic stimulation leads to an increase in local CBF (n = 4).

**Conclusions:** These findings suggest that NK1R/nNOS-expressing cortical inhibitory interneurons act as local integrators of neural activity to mediate changes in CBF. This insight into the neural basis of CBF is fundamental to understanding the pathogenesis of cerebrovascular diseases and for the development of therapeutics targeting cerebral perfusion.

#### **T-05: PATHOLOGICAL EFFECTS OF SORAFENIB ADMINISTRATION TO SPRAGUE-DAWLEY RATS**

Yi-Hui Su, Yew-Min Tzeng, Jiunn-Wang Liao

**Background:** Sorafenib (BAY43-9006) is an oral tyrosine kinase inhibitor, approved for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma by FDA. The common side effects in humans include gastrointestinal disturbance, hypertension, bleeding, etc. Moreover, long-term sorafenib-intake may lead to multi-organ toxicity and could be life-threatening.

**Objective:** To evaluate the possible pathological changes in rats following 28-day administration of sorafenib.

**Methods:** Sprague-Dawley rats were separated into two groups, 10 in each group of 5 male and 5 female rats. Both the groups were treated by oral gavage for 28 consecutive days. Group 1 animals were administered olive oil and Group 2 animals were administered 7.5 mg/kg BW sorafenib. Clinical examination, clinical pathology (hematology, serum chemistry and urinalysis), necropsy and histopathology were conducted at the end of the trial.

**Results:** In the sorafenib-treated group, male rats showed body weight loss and decrease of food consumption, and some of the rats presented rough hair in appearance. Clinical pathological examination revealed slightly elevated liver-associated enzymes, indicated damage to cells in the liver. Diffuse epiphysis hypertrophy (5/5 in male and 5/5 in female rats) and proliferation, diffuse hypocellularity in the bone marrow (3/5 in male rats), diffuse atrophy in the spleen (5/5 in male rats) and increase in the number of follicles in the ovaries (5/5 in female rats) were found under histopathological examination.

**Conclusion:** Multi-organs toxicity of the liver, bone marrow, growth plate, spleen and ovaries were found in sorafenib treated rats at the dose 7.5 mg/kg BW orally for 28 days.

#### **T-06: RETROSPECTIVE ANALYSIS OF FUNGAL RHINITIS IN SPRAGUE-DAWLEY RATS: SEX DIFFERENCES IN INFECTION FREQUENCY AND LESION LOCATION IN NASAL CAVITY**

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**Conclusions:** While the overall incidence is low, the incidence varied between studies, and fungal rhinitis can be a significant cause of morbidity in aged rats. The consistently observed wide variance in incidence and location between males and females is not readily explained and warrants further investigation.

#### **T-07: INVESTIGATION OF SILVER DEPOSITION AND INFLAMMATORY LESION IN SELECTED ORGANS OF STRANDED CETACEANS**

Wen-Ta Li, Bang-Yeh Liou, Wei-Cheng Yang, Meng-Hsien Chen, Hui-Wen Chang, Victor Fei Pang, Chian-Ren Jeng

**Background:** Silver nanoparticles (AgNPs) have been extensively used in commercial products and released into the environment. Therefore, AgNPs have been considered a potential source of Ag contamination and raise the public concern about the environmental toxicity. Cetaceans as apex predators of the ocean have been shown to have a relatively high level of Ag in their bodies. Although the immunotoxicity of AgNPs have been demonstrated in cetaceans, the correlation between Ag deposition and the lesion in cetaceans is poorly understood.

**Materials and Methods:** Tissue samples, including central nervous system, lung, heart, liver, kidney, gastrointestinal tract, were collected from 54 stranded cetaceans. The correlation between the lesion variables and Ag concentrations in the liver of each individual specimen were investigated. The lesion score was obtained as: absence [score: 0] or presence [score: 1] of inflammatory lesions in each organ, and total lesion scores were the accumulation from six organs of each individual.

**Results:** Total lesion scores (TLSs) and the periportal hepatitis were positively correlated with Ag concentrations (Spearman's rho;  $r = 0.2731$ ;  $p = 0.0453$  and  $r = 0.3752$ ;  $p = 0.0052$ ). Although only an increased trend of Ag concentrations was noted with increased TLSs, Ag concentrations were significantly higher in stranded cetaceans with periportal hepatitis (Mann-Whitney U test;  $p = 0.0056$ ).

**Conclusion:** This study demonstrated the possible negative health effects of Ag in cetaceans, suggesting that 1) Ag is direct-acting toxicants and inducing damage to the periportal hepatocytes and inflammatory response, and/or 2) the periportal hepatitis is induced by an autoimmune response due to the immunotoxicity of Ag.

#### **T-08: OUTBREAK OF KIKUYU (*Pennisetum clandestinum*) TOXICOSIS IN CATTLE IN NEW SOUTH WALES, AUSTRALIA**

Thomas Westermann, Sarah Gestier, Zoe Spiers, Mark Hazelton, Sam Gilchrist, Keith Walker, Jim Kerr, Lyndell Stone, Digby Rayward, Pedro Pinczowski, Leah Manning

From late summer to early fall in 2018 in Australia, an outbreak of kikuyu (*Pennisetum clandestinum*) toxicosis in cattle killed more than 63 animals. The Elizabeth Macarthur Agricultural Institute received submissions from nine affected beef and dairy properties located in central and northern coastal regions of New South Wales. This work describes epidemiological, clinical and diagnostic aspects of kikuyu toxicosis in cattle, which occurs sporadically in Australia, New Zealand and South Africa. Kikuyu toxicosis

is typically associated with recent introduction to lush kikuyu pasture following drought breaking rains, although pathogenesis is not well understood. Death in this outbreak occurred suddenly, or up to 36 hours after onset of clinical signs, most commonly including hypersalivation, polydipsia, sham drinking, bruxism, depression and recumbency. Herd morbidity rates ranged from 2-10% and mortality rates ranged from 6-20%. Cattle necropsied commonly had signs of severe dehydration, massive ruminal distension with frothy green fluid and grass material, and pallor and sloughing of ruminal, reticular and omasal mucosa. When evaluated, rumen fluid pH ranged from 6.5 to 8.0, and serum and aqueous humour biochemistry commonly indicated muscle injury, dehydration and inflammation. Histopathological changes were seen predominantly in forestomachs, and were consistent with marked, necrosuppurative rumenitis, reticulitis and omasitis, in 90% (9/10), 100% (5/5) and 91% (10/11) of cases, respectively. The etiologic diagnosis of kikuyu toxicosis was made based on consistent epidemiology, clinical signs, biochemical results and gross and histopathologic lesions.

## Natural Disease Focused Scientific Session I

November 4, 2018 | 8:00 AM – 12:00 PM

Session Chair: Daniel R. Rissi, DVM, PhD, DACVP, University of Georgia, Athens, GA

November 4, 2018

8:45 AM – 9:00 AM

### **CANINE EPENDYMOMA: DIAGNOSTIC CRITERIA AND HISTOLOGIC PITFALLS**

Andrew Miller, Jennifer Koehler, Taryn Donovan, Jennifer Stewart, Brian Porter, Daniel Rissi, Simon Priestnall, Frances Schulman

**Background:** Reports of canine ependymoma are generally restricted to single case reports with tumor incidence estimated at ~3% of primary central nervous system tumors (CNS) in the dog. While most commonly reported in the lateral ventricle, they can occur anywhere in the ventricular system. Rosettes and pseudorosettes are a common histologic feature; however, this pattern can be mimicked by other CNS neoplasms.

**Objective:** This study aims to define the key histopathologic and immunohistochemical characteristics of canine ependymoma.

**Methods:** We performed a retrospective database search of eight institutions for cases of canine ependymoma. Thirty-seven cases were identified and a complete histologic review of all cases was performed. Of these thirty-seven cases, twenty-four candidate cases were further subjected to a panel of immunohistochemical stains.

**Results:** 5/37 cases were conclusively identified as ependymoma. Locations included lateral ventricle (3/5), 3<sup>rd</sup> ventricle (1/5), and mesencephalic aqueduct (1/5). Histologic features included rosettes (5/5), pseudorosettes (5/5), clear cell differentiation (1/5), ependymal canals (2/5), blepharoplasts (1/5), ciliated cells (1/5), necrosis (3/5), and high nuclear:cytoplasmic ratio (5/5). Immunoreactivity for GFAP (4/4) and CKAE1/3 (3/4) was found in pseudorosettes, rosettes, and single neoplastic cells. Diffuse, but variable, S100 immunoreactivity was detected in 3/4 cases. Olig2 intranuclear immunoreactivity was sparse in less than 1% of the neoplastic cells (3/3). Tumors that mimicked the characteristic ependymal pseudorosettes included astrocytoma, choroid plexus tumor, papillary meningioma, pituitary gland tumor, and oligodendroglioma.

**Conclusions:** Canine ependymoma is an extremely rare neoplasm of which the diagnostic features often overlap with other primary CNS neoplasms.

November 4, 2018

9:00 AM – 9:15 AM

### **CHARACTERIZATION OF SYSTEMIC AMYLOID A AMYLOIDOSIS IN STEJNEGER'S BEAKED WHALES (MESOPLODON STEJNEGERI)**

Shotaro Nakagun, Kenichi Watanabe, Yuko Tajima, Tadasu Yamada, Noriyuki Horiuchi, Yoshiyasu Kobayashi

**Background:** Stejneger's beaked whales (*Mesoplodon stejnegeri*) are one of the lesser known species of cetaceans, with little information available on their population status or incidence of diseases. Recent pathologic investigations on stranded and bycaught wild cetaceans around Hokkaido, Japan, have revealed an unusually high prevalence of amyloidosis in this species, warranting further attention.

**Objective:** The objective of this study was to characterize the amyloidosis of Stejneger's beaked whales by retrospective analysis by examining animals stranded in Japan between 1994 and 2018. We describe the histologic, immunohistochemical and proteomic features of the amyloidosis occurring in this species.

**Methods:** Histopathologic sections of various tissues from 36 individuals were examined, which included whales of both sex and all age groups. Representative tissues from each animal were stained with Congo red and immunohistochemistry for amyloid A, for the definitive diagnosis of amyloidosis. Furthermore, in order to determine the type of the deposited amyloid, amyloid was extracted from the liver by homogenization with water and centrifugation at high speed. The extracts were electrophoresed through SDS-polyacrylamide gel and a subsequent western blotting for amyloid A was performed.

**Results:** Out of the 36 whales examined, 11 were histologically diagnosed as systemic amyloidosis. Immunohistochemistry and western blotting confirmed the nature of the amyloid as amyloid A.

**Conclusions:** Although the cause of systemic amyloid A amyloidosis in Stejneger's beaked whales remains uncertain, the unusually high prevalence (over 30%) is of concern for the species' survival. Further research is needed in elucidating the pathogenesis of this debilitating proteopathy.

November 4, 2018  
9:15 AM – 9:30 AM

## **AN OUTBREAK OF NECROTIZING DERMATITIS IN MOOSE CALVES DUE TO A NOVEL CERVIDPOXVIRUS**

Anibal Armien, Tiffany Wolf, Sunil Mor, Terry Ng, Sagar Goyal, James Rasmussen

**Background:** There is increasing evidence of the emergence of novel poxviruses. Recently, we described *Deerpox virus* (DPV) infection in a goitered gazelle, representing the first report of spontaneous infection and clinical disease in a species outside of the *Cervidae* family. The DPV is a sole member of the newly created *Cervidpoxvirus* family and this virus was first isolated from wild mule deer (*Odocoileus hemionus hemionus*) fawns.

**Objective:** To describe spontaneous infection by a novel *Cervidpoxvirus* in the North American moose (*Alces americanus*).

**Results:** Three, 4-month-old moose calves presented with necrotizing granulomatous multifocal dermatitis in the head and legs. At transmission electron microscopy, application of both negative and positive contrast on subacute lesions, the virus was

consistently found in debris, necrotic epidermal cells and in large number of reactive neutrophils. On negatively contrasted preparations of fresh crust and skin lesions, this moose poxvirus presented brick-shape morphology and displayed both mulberry (M) and capsular (C) forms. All stages of poxvirus morphogenesis (crescents, spherical immature particles, mature particles, and enveloped mature virus) were observed. Vero CCL-81 cells infected with this novel Cervidpoxvirus confirmed that its morphogenesis is similar to the morphogenesis model proposed for the prototype vaccinia virus. The genome of moosepox virus was obtained; the entire coding region including its 170 putative genes are sequenced and annotated. The sequence length of the moosepox virus (MPV, GenBank MG751778) is 164,258 bp,

**Conclusions:** This study provides the biological characterization of a new *Cervidpoxvirus* attained through *in vivo* and *in vitro* ultrastructural analyses.

November 4, 2018

9:30 AM – 9:45 AM

## **EMERGENCE OF RABBIT HEMORRHAGIC DISEASE VIRUS 2 IN BRITISH COLUMBIA, CANADA**

Heindrich Snyman, Tomy Joseph, Helen Schwantje, Aruna Ambagala, Glenna McGregor, Ann Britton, Stephen Raverty, Robert Cooper, Carissa Embury-Hyatt, Kathleen Hooper-McGrevy, Shawn Babiuk, Jane Pritchard

**Background:** Rabbit hemorrhagic disease (RHD) is a contagious and fatal disease of domesticated and wild European rabbits (*Oryctolagus cuniculus*). RHD virus (RHDV) is a Lagovirus of the family Caliciviridae. Three major viral subtypes are recognized: RHDV (classical RHDV), a related antigenic variant RHDVa, and RHDV2 (RHDVb). RHDV2 was first identified in France in 2010, and since then has spread throughout Europe where it has become the dominant circulating strain.

**Objectives:** To characterize an outbreak of RHD in rabbits.

**Results:** In February 2018, sudden high mortality was reported in a large population of feral European rabbits on the Vancouver Island University Campus (Nanaimo, British Columbia). Malicious poisoning was initially suspected and three dead rabbits were submitted to the Animal Health Centre for further evaluation. Rabbits had rare pulmonary and epicardial hemorrhages and histologically had widespread acute hepatocellular necrosis, lymphocyte karyorrhexis, and renal glomerular thrombosis. Tissue samples tested positive for RHDV by PCR and was confirmed by the NCFAD (Winnipeg, Manitoba). The virus (RHDV-2-BC-2018-1) was isolated by rabbit inoculation and the whole genome sequenced. The RHDV-2-BC-2018-1 isolate showed only 93% identity to a RHDV2 isolate (RHDV2-QC-2016) from a hobby farm in Mont-Joli, Quebec in August 2016. Additional cases of RHD were subsequently reported on Vancouver Island and the BC mainland involving various feral populations, commercial rabbitries, and shelter populations and the outbreak is ongoing.

**Conclusions:** This is the third confirmed diagnosis of RHD in Canada, the first in BC, and by far the largest reported outbreak of RHD in North America.

November 4, 2018

9:45 AM – 10:00 AM

### **BREED SPECIFIC DIFFERENCES IN MOLECULAR FEATURES AND CLINICAL OUTCOME IN CANINE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

Emily Rout, Julia Labadie, Robert Burnett, Janna Yoshimoto, Anne Avery

**Background:** Human B-cell chronic lymphocytic leukemia (B-CLL) demonstrates marked heterogeneity in clinical outcome and pathophysiology, but little is known about heterogeneity within canine B-CLL. An important prognostic factor in human B-CLL is immunoglobulin heavy chain variable (IGHV) gene mutation status. Human B-CLL patients with unmutated IGHV genes, having not undergone somatic hypermutation, have more aggressive disease than patients with IGHV genes that have undergone somatic hypermutation.

**Objectives:** Examine IGHV mutation status, clinical outcome and gene expression among canine B-CLL patients, comparing small-breed dogs (over-represented in B-CLL) and Boxer dogs.

**Methods:** We sequenced the IGHV genes of 55 canine B-CLL patients, including 36 non-Boxers and 19 Boxers, and used sequence homology with the reference genome to determine mutation status. Medical records from 42 small-breed and 18 Boxer B-CLL patients were reviewed. We used RNA-Seq to examine gene expression profiles among canine B-CLL cases, diffuse large B-cell lymphoma cases and normal B cells, including Boxer B-CLL (n=4) and small-breed B-CLL (n=8) cases.

**Results:** Boxer dogs preferentially used unmutated IGHV genes (79% of cases), while only 25% of non-Boxer cases were unmutated. Median overall survival was significantly shorter in Boxers (MST=5 months) than small-breed dogs (MST=19 months). Gene expression analysis segregated B-CLL cases from DLBCL cases, and showed heterogeneity amongst B-CLL samples. Differentially expressed genes in B-CLL versus control B cells were enriched for B-cell receptor and NF-KB signaling pathways.

**Conclusions:** Canine B-CLL has heterogeneous molecular features and clinical outcome. Boxers appear to preferentially use unmutated IGHV genes and have more aggressive disease.

November 4, 2018

10:30 AM – 10:45 AM

### **DETERMINATION OF TREATMENT FAILURE FOR FIP INFECTED CATS TREATED WITH ANTICORONAVIRAL THERAPY TO FACILITATE THE SUCCESS OF SECOND GENERATION THERAPY**

Molly Liepnieks, Brian Murphy, Michel Perron, Michael Bannasch, Elizabeth Montgomery, Eisuke Murakami, Hongwei Liu, Niels Pedersen

**Background:** Utility of GS-441524 was evaluated in 31 naturally feline infectious peritonitis virus (FIPV) infected client-owned cats. 26 cats completed one or more

rounds of treatment. To date (10-13 months post-study enrollment), 24 cats remain healthy. Seven cats were euthanized.

**Objective:** Determine the cause of treatment failure in naturally FIPV infected cats treated with anticoronaviral therapy in order to facilitate the success of second generation antiviral therapy.

**Methods:** Necropsies were performed on 6/7 cats that were euthanized 2-23 days following trial enrollment; one cat was lost to follow-up. Full necropsy with histopathologic examination was performed. FIPV immunohistochemistry was performed on selected tissues. Viral RNA transcripts were quantified by qRT-PCR from ascites fluid when available.

**Results:** Four cats were found to have FIPV lesions; three in the central nervous system and one restricted to the abdomen. No evidence of FIPV infection was identified in two cats; one with a gastric perforation and one with thromboembolic disease secondary to congenital hypertrophic cardiomyopathy (HCM). Of four cats euthanized due to FIPV infection, three had evidence of ongoing viral replication by IHC and/or qRT-PCR.

**Conclusions:** To date, 92% of cats completing treatment have been cured and data supports drug-associated inhibition of viral replication in vivo. Cats failing treatment often failed early, demonstrated ongoing viral replication, and neurologic involvement. Ongoing viral replication may represent viral resistance or host factors (inability to activate prodrug). Neurologic disease may require more aggressive or multimodal treatment. Additionally, early failures may represent more severe disease at time of treatment initiation.

November 4, 2018

10:45 AM – 11:00 AM

### **BETA-MANNOSIDOSIS IN MIXED-BREED SIBLING DOGS**

Gayle Johnson, Gary Johnson, Dennis OBrien, Ana Kolicheski, Chris Levine

**Background:** Deficiency of  $\beta$ -mannosidase enzyme activity results in clinical neurological disease. Early onset, demyelinating  $\beta$ -mannosidosis occurs in cattle and goats, but there is a later onset, and milder clinical signs described in people.

**Objective:** Characterize two cases of  $\beta$ -mannosidase in dogs.

**Methods:** Two female miniature poodle-Maltese crossbred dogs were presented at 15 and 17 months of age for progressive cerebellar signs, seizures, dementia and paresis. Prior to necropsy, magnetic resonance imaging of both dogs revealed enlarged lateral brain ventricles. At necropsy, fixed and refrigerated tissues were collected and submitted for testing. Whole genome sequencing was done, with approximately 28-fold average genomic coverage. Based on these results, frozen brain was tested for  $\beta$ -mannosidase activity.

**Results:** Enlargement of lateral ventricles and a rounded liver capsule were observed grossly. Larger neurons with defined, unstained cytoplasmic vacuoles were found histologically. Purkinje neurons were reduced in number in the cerebellum. Vacuolated cells were also found in spinal cord, visceral organs and eye. Ultrastructurally, neuronal cytoplasmic vacuoles were membrane-bound, but had minimal visible internal content. A C-to-A transversion in exon 14 of *MANBA*, predicted a Try669Cys amino acid substitution in the  $\beta$ -mannosidase gene. Detectable  $\beta$ -mannosidase activity was not found in frozen brain.

**Conclusions:**  $\beta$ -mannosidosis occurred in 2 of 6 members of litter of mixed breed dogs, and had a phenotype that was intermediate between that found in goats and cattle, and that commonly found in people. Unfortunately, the breeder of the dogs refused genetic testing of parents or siblings.

November 4, 2018

11:00 AM – 11:15 AM

### **PATHOLOGIC AND MOLECULAR DIAGNOSIS OF YELLOW FEVER IN NEOTROPICAL NON-HUMAN PRIMATES, BRAZIL**

Natália Fernandes, Juliana Guerra, Rodrigo Réssio, Cinthya Cirqueira, Silvia Iglesias, Cristina Kanamura, Josué Díaz-Delgado, Isis Rizkallah, Karolina Beraldo, Lidia Kimura, Mariana Cunha, Leonardo Araújo, José Catão Dias

**Background:** Epizootics of yellow fever (YF) in non-human primates (NHPs) are sentinel events that may indicate YF virus presence and dynamics, and often precede YF-human fatalities. Surveillance and diagnosis of YF in NHPs are pivotal to deploy preventive and contingency measures.

**Objectives:** To report prevalence of YF and evaluate correlation between histopathologic, immunohistochemical (IHC) and molecular diagnoses in NHPs analyzed at the Pathology Center of the Adolfo Lutz Institute (a Brazilian reference laboratory) in 2017.

**Methods:** Liver tissues of 2,171 NHPs were examined microscopically and immunohistochemically. Fresh frozen (FF) or formalin-fixed, paraffin-embedded (FFPE) samples (including liver and others) of 1,744 were submitted to real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis.

**Results:** The liver of 626 (28.83%) NHPs was positive for YF virus by IHC. From these, 577 (92.17%) NHPs exhibited typical YF histopathologic liver lesions. Autolysis precluded microscopic examination in 46 (4.15%) NHPs. The agreement between histopathologic and IHC diagnostic methods in liver samples was good (kappa:0.980; CI: 0.9707-0.9895;  $p < 0.001$ ). Furthermore, 28 (1.29%) NHPs were positive only by FF-RT-qPCR. One case was positive by IHC and RT-qPCR on FFPE-liver, but negative in multiple FF tissues. The agreement between IHC and RT-qPCR was also good (kappa: 0.946; CI: 0.927-0.966;  $p < 0.001$ ).

**Conclusions:** Parallel histopathologic and IHC analysis of liver tissue of NHPs remains a highly reliable tool for YF diagnosis. Some cases may benefit from additional RT-qPCR analysis in FFPE tissues when FF samples were not available or yielded a discordant result.

## Natural Disease Focused Scientific Session II

November 4, 2018 | 1:30 PM – 5:00 PM

Session Chair: Daniel R. Rissi, DVM, PhD, DACVP, University of Georgia, Athens, GA

November 4, 2018

2:15 PM – 2:30 PM

### **BLUETONGUE VIRUS (BTV) INFECTION IN RAMS INDUCES THE ACTIVATION OF TYPE-I INTERFERON RESPONSE RESULTING IN TESTICULAR DEGENERATION**

Davide Pintus, Giantonella Puggioni, Giorgio Meloni, Rosario Scivoli, Eleonora Melzi, Angela Rocchigiani, Daniela Manunta, Annalisa Oggiano, Massimo Palmarini, Ciriaco Ligios

**Background:** Testicular degeneration with destruction of the tubular germinative cells has been often described in human and other animals, as a result of different viral infections. However, the pathogenesis of these infections leading to infertility conditions is little known and studies have only referred to murine model. Interestingly, the recent Zika virus epidemic highlighted the possibility that arboviruses may negatively affect the male reproductive tract.

**Objective:** In this study, we focused on bluetongue virus (BTV), the causative agent of bluetongue disease, for investigating the pathological consequences of arbovirus infection on male reproductive tract.

**Methods:** By using 2 different isolates of BTV serotype 1 collected from naturally infected sheep, during 2006 (BTV-1<sub>IT2006</sub>) and 2013 (BTV-1<sub>IT2013</sub>) epidemics in Sardinia (Italy), 2 groups of 6 rams were infected and serially euthanized for pathological and virological investigations.

**Results:** Testicular degeneration and azoospermia was induced in these rams with either BTV-1<sub>IT2006</sub> or BTV-1<sub>IT2013</sub> isolates, although the former was significantly less virulent. Testicular lesions in BTV-infected rams were due to viral replication in endothelial cells of the intertubular vessels, resulting in stimulation of a type-I interferon (IFN) response, reduction of testosterone biosynthesis by Leydig cells, and destruction of Sertoli cells of the blood-testis barrier in more severe cases.

**Conclusions:** Unlike other gonadotropic viruses, BTV induces testicular degeneration and disruption of spermatogenesis by replicating solely in the endothelial cells of the intertubular vessels and leading to type-I IFN response. This study shows that a naturally occurring arboviral disease can cause testicular degeneration and affect male fertility.

November 4, 2018

2:30 PM – 2:45 PM

### **CHARACTERIZATION OF THE TRANSCRIPTIONAL PROGRAM OF FELINE INJECTION-SITE SARCOMA**

Qi Wei, Stephen Ramsey, Maureen Larson, Christiane Löhr

**Background:** Soft tissue sarcomas are among the most common skin tumors in cats. A highly aggressive subgroup, feline injection-site associated sarcoma (FISS), develops at sites of previous injection. The pathogenesis of FISS is poorly understood and treatment options are limited.

**Objective:** To analyze mRNA-seq data of FISS tumors vs. normal tissues to elucidate biological processes and molecular pathways that are associated with the pathogenesis of FISS.

**Methods:** Using high-throughput short-read paired-end sequencing, FISS tumors vs. normal tissue were comparatively profiled for cancer/normal gene expression levels. Post-hoc analyses identified altered molecular pathways, putative novel FISS-specific transcripts, potential somatic copy number alteration (SCNA) in FISS.

**Results:** Our research found transcriptional alterations in FISS compared to normal tissue, identified substantial overlap between the set of human orthologs of cat genes with altered transcriptional abundances in FISS with sets of known human tumor suppressor genes (TSGs) and oncogenes, and identified a 10 Mbp region of potential FISS SCNA whose syntenic human genomic region has a recurrent SCNA in human sarcomas. Our results closely align with findings in human and canine sarcomas and confirm a SCNA previously identified in FISS by other means.

**Conclusions:** To the best of our knowledge, this is the first mRNA sequencing-based transcriptome profiling study of FISS and any cancer in cats.

November 4, 2018

2:45 PM – 3:00 PM

## **AN EPIDEMIOLOGIC INVESTIGATION OF OPHIDIAN NIDOVIRUSES IN CAPTIVE SNAKES**

Laura Hoon-Hanks, Pia Bartolini, Susan Fogelson, Sean Perry, Robert Ossiboff, Edward Dubovi, Jim Wellehan, Mark Stenglein

**Background:** Nidoviruses are a large and diverse order of viruses that include notable human and veterinary pathogens. Recently, novel nidoviruses in the subfamily *Torovirinae* have been found in snakes, lizards, cattle, and nematodes, and those found in vertebrates have often been associated with severe respiratory disease. The diversity of the full host ranges of these viruses is unknown, including within ophidians (snakes). Previous studies have found ophidian nidoviruses in pythons and boids, but a large-scale study including non-python and non-boid species has not been performed.

**Objectives:** Our study targeted snake collections with mixed snake species, including pythons, boids, colubrids, elapids, and viperids.

**Methods:** Collections had either epidemic, endemic, or unknown ophidian nidovirus status. Infection status was assessed through consensus PCR, sanger sequencing, and Illumina sequencing.

**Results:** Preliminary results indicate high infection rates in the superfamily *Henophidia*, which contains *Pythonidae* and *Boidae* families, with significant morbidity and mortality often seen in positive collections. Nidoviruses were also detected in *Colubridae* hosts, but the significance of these infections is currently unknown. *Elapidae* and *Viperidae* hosts were negative, although sample size was low for these families. Examined collections often had multiple circulating ophidian nidoviruses and transmission events of a single isolate were tracked between different species of snakes, indicating some degree of host range plasticity.

**Conclusions:** Further research is warranted to determine the clinical significance of different ophidian nidoviruses in different host species, and the influence of environmental, host, and virus variables on disease manifestation.

November 4, 2018

3:30 PM – 3:45 PM

### **WHOLE GENOME SEQUENCING IN RELATING ANIMAL ILLNESS AND PET FOODBORNE INFECTIOUS AGENTS**

Jennifer Jones, Olgica Ceric, Laura Goodman, Arthur Pightling, Sarah Nemser, Renate Reimschuessel, Lee Anne Palmer, Lauren Carey, Elizabeth Edwards, Mark Glover, Jackie Queen, April Hodges, Sonya Lambkin, Neiunna Reed-Jones, David Rotstein

**Background:** Pet foodborne pathogens including *Salmonella*, *Listeria monocytogenes*, and Shiga toxin-producing *E. coli* present health risks to pets and humans and can be isolated with routine pathogen surveillance. Pulse field gel electrophoresis (PFGE), serotyping, and multiple-locus variable number tandem repeat analysis have been the mainstays of comparing isolates and identifying possible illness clusters. Whole genome sequencing (WGS) is replacing PFGE because of its greater discriminatory power in comparing single nucleotide polymorphisms (SNP).

**Objective:** Present the use, methods, and findings from WGS in two pet food illness investigations.

**Methods:** Two case investigations conducted in 2016-2017 of illness and death from *Salmonella* spp. and/or *Listeria monocytogenes* with exposure to unrelated brands (Food 1 and Food 2) of raw commercial pet food were compared to isolates from the same brands in 2018. Core genome multi-locus sequence typing was utilized for sequencing and compared using the National Center for Biotechnology Information's Pathogen Detection Isolates Browser to evaluate if the animal and food isolates were related by the determination of fewer than 10 SNP differences.

**Results:** The 2018 Food 1 isolates were genetically identical to 2016 food and animal isolates; the 2018 Food 2 isolates were genetically unrelated to the 2016 food and animal isolates.

**Conclusions:** WGS comparisons provides a means to identify human or animal foodborne illness outbreaks, enhancing public health responses, enhancing regulatory actions, and elevating clinical information beyond isolation.

November 4, 2018

3:45 PM – 4:00 PM

### **ENCEPHALOMYOCARDITIS VIRUS IN CAPTIVE AUSTRALIAN MARSUPIALS**

John Mackie, Andrew Hill, Michael Pyne, Claude Lacasse

**Background:** Encephalomyocarditis virus (EMCV) is an RNA virus, genus *Cardiovirus*, family *Picornaviridae*. It occurs worldwide and can infect a wide range of mammals. Rodents are the reservoir hosts and shed the virus in feces and urine. EMCV causes sporadic, often fatal disease, mainly in swine and nonhuman primates and occasionally in zoo animals.

**Objectives:** Characterize an outbreak of EMCV infection in captive Australian marsupials.

**Results:** In 2017, 5 of 43 adult eastern grey kangaroos (*Macropus giganteus*) died over a 10-day period at a zoological garden in Queensland, Australia. Most were found dead. Gross findings included pale foci throughout the myocardium. Histologically, there was myocardial necrosis with lymphohistiocytic inflammation and patchy mineralization. EMCV was detected in fresh heart by real-time PCR. There were no cases in six other species of macropods kept in adjacent enclosures. In response to the outbreak, a stricter rodent control program was implemented. One further case in an eastern grey kangaroo occurred 6 months later. This zoo routinely administers inactivated EMCV vaccine to some exotic mammals and one endangered species of macropod, but not eastern grey kangaroos. Around the same time, another zoo (approximately 180 km away) experienced a similar outbreak, with sudden death in a quokka (*Setonix brachyurus*), a common wombat (*Vombatus ursinus*) and two eastern grey kangaroos. EMCV was detected in fresh heart by real-time PCR.

**Conclusions:** This report extends the species range for fatal EMCV infection. EMCV is used as a model of acute viral myocarditis (Cocksackievirus myocarditis in humans) and diabetes mellitus.

November 4, 2018

4:00 PM – 4:15 PM

### **IMMUNOHISTOCHEMICAL FEATURES OF CANINE INTRACRANIAL EMBRYONAL NEOPLASIA: 10 CASES**

Elena Demeter, Jennifer Koehler, Brian Porter, Daniel Rissi, Andrew Miller

**Background:** Embryonal tumors are a diverse set of neoplasms and include those primary to the central nervous system (CNS) (e.g. medulloblastoma and primitive neuroectodermal tumors) as well as those occurring adjacent to and invading into the CNS (e.g. olfactory neuroblastoma). They likely derive from germinal neuroepithelial cells and can differentiate along divergent neuroectodermal lineages. In human neuropathology, embryonal CNS tumors are diagnosed through a diverse set of immunohistochemical stains, including microtubule-associated protein 2 (MAP2), synaptophysin, neuron specific enolase (NSE), neurofilament, and neuronal nuclear

antigen (NeuN). In dogs, CNS-associated embryonal tumors are uncommon, and their immunohistochemical profile is poorly characterized.

**Objective:** Determine the immunohistochemical features of canine intracranial embryonal tumors.

**Methods:** Ten cases of canine intracranial embryonal neoplasia (medulloblastoma (4/10), olfactory neuroblastoma (2/10), and embryonal tumor, NOS (4/10)) were stained for MAP2, synaptophysin, NSE, neurofilament, NeuN, and oligodendrocyte transcription factor 2 (Olig2). Staining patterns were analyzed for distribution, localization, and intensity.

**Results:** For all cases, MAP2 and synaptophysin had multifocal to diffuse, strong cytoplasmic immunoreactivity that was typically restricted to more differentiated, neuronal-like cells. All cases had only rare to less than 10% of cells that had immunoreactivity to NSE, NeuN, and Olig2. All cases lacked immunoreactivity for neurofilament.

**Conclusions:** The staining pattern supports the need for an immunohistochemistry panel in diagnosing canine embryonal CNS tumors. MAP2 and synaptophysin are the most useful in aiding the diagnosis of CNS embryonal tumors coupled with a lack of the diffuse Olig2 immunoreactivity typical of a glioma.

## Natural Disease Focused Scientific Poster Session

### N-01: PROSTAGLANDIN E RECEPTOR EP3 EXPRESSION IN FELINE ORAL SQUAMOUS CELL CARCINOMA

Nicole Kaiser, Kathleen Jones, Shanon Martinson, Chelsea Martin

**Background:** Feline oral squamous cell carcinoma (FOSCC) is an extremely aggressive malignant neoplasm and is the most commonly diagnosed tumor of the oral cavity in cats. Prostaglandin E2 (PGE2) has been shown to play an important role in the pathogenesis of cancer and is often a target in treatment. However, adverse effects associated with cyclooxygenase (COX) 1 and 2 inhibitors can limit their use in some patients. Targeting elements further along the inflammatory cascade, such as prostaglandin E2 (PGE2) receptors, may circumvent these harmful side effects, but the expression of prostaglandin receptors in FOSCC is currently unknown.

**Methods:** Forty formalin-fixed paraffin embedded samples of FOSCC were evaluated for expression of the PGE2 receptor EP3 using immunohistochemistry, and were compared to twenty samples of normal feline oral mucosa. Staining intensity was scored using a visual grading system for both the nuclear and cytoplasmic compartments, which incorporated staining intensity and proportion of positive cells.

**Results:** EP3 receptor expression was lower in FOSCC samples compared to normal oral mucosa from cats without FOSCC. The average expression score for FOSCC epithelial cell cytoplasm was 11.0, compared to 14.4 in normal feline oral epithelium ( $p < 0.05$ ). There was no difference in nuclear localization of EP3.

**Conclusions:** These results imply that EP3 downregulation may play a role in development and progression of FOSCC. Thus, therapeutic approaches that preserve or augment EP3 receptor signaling may be of benefit to cats with FOSCC. To our knowledge, this is the first study demonstrating EP3 receptor expression in feline tissues.

### N-02: IMMUNOHISTOCHEMICAL EVALUATION OF CD25 EXPRESSION IN CANINE LYMPHOMA

William Sills, Andrew Miller, Kristy Richards

**Background:** Diffuse large B-cell lymphoma (DLBCL) represents the most common lymphoid neoplasm in dogs, and shares many clinicopathologic and molecular similarities to human DLBCL. Expression of the interleukin-2 receptor  $\alpha$ -chain (CD25) has shown prognostic significance in human DLBCL.

**Objective:** The objectives in this study were to generate a canine lymphoma tissue microarray (TMA) for immunophenotyping canine DLBCL and to determine the histologic features that correlate to CD25 expression.

**Methods:** Cases of canine DLBCL, T-cell lymphoma (TCL), or reactive lymphoid hyperplasia (RLH) were selected for the microarray. An anatomic pathology resident and board-certified veterinary pathologist assessed each core, blinded to the original

diagnoses. A validated CD25 antibody was applied and expression was scored on a semi-quantitative scale (0-5).

**Results:** Assessing the TMA, 22/24 consensus diagnoses concurred with the original diagnosis (2 RLH were incorrectly identified as TCL). CD25 expression was detected at high levels (grade 4/5 or 5/5; >60% of total neoplastic population) in 10/12 DLBCL and 1/6 T-cell lymphomas. CD25 expression in the remaining 2 DLBCLs was <10% in neoplastic cells (grade 0/5). The expression was more variable among the remaining 5/6 TCLs, ranging from grades 0/5 to 2/5. Similar variability was observed among RLH, ranging from grades 2/5 to 5/5.

**Conclusions:** We confirmed expression of CD25 in canine lymphoid tissue and lymphoma, including a subset of DLBCL expressing high levels of CD25. Tissue microarray represents a useful tool for evaluating molecular markers in multiple samples simultaneously, and will provide a platform for establishing prognostic implications of CD25 in DLBCL.

### **N-03: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF INFLAMMATORY CELLS IN CANINE GLOMERULAR LIPIDOSIS**

Katherine Watson, Peter Moore, Rachel Cianciolo, Kristy Harmon, F. Mohr

**Background:** Glomerular lipidosis, a risk factor associated with progression to glomerulosclerosis, is characterized by a segmental change in the mesangium of the glomerular tuft with aggregation of lipid-laden foam cells. Mechanistic theories on the development of this disorder hypothesize that podocyte injury results in disordered lipid metabolism with endothelial and mesangial production of proinflammatory cytokines that initiate macrophage recruitment and foam cell transformation within the glomerulus. Lipid-laden foam cell formation begins when phagocytes ingest and process apolipoprotein B-containing lipoproteins (apoB-LPs) in part by scavenger receptors. ApoB-LP-initiated modifications to the endothelium and macrophages results in increased production of chemokines and cytokines, which recruits additional inflammatory cells to the glomerulus.

**Objectives:** Our objective was to use immunohistochemistry to characterize glomerular inflammatory and foam cell surface markers of dogs with glomerular lipidosis.

**Methods:** Surface antigens on lipid-laden foam cells were detected on cryosections using monoclonal antibodies specific for canine leukocyte antigens sourced from the Leukocyte Antigen Biology Laboratory, University of California, Davis. A limited number of antibodies recognized formalin-denatured epitopes following heat-based antigen retrieval.

**Results:** The inflammatory and foam cells that aggregate within glomerular tufts are strongly immunopositive for CD45, CD204, CD11b, CD11c, CD11d and CD1 (9H11). Cells were moderately immunopositive for MHC-II.

**Conclusions:** Based on the immunohistochemical findings, the accumulation of inflammatory and foam cells in glomerular lipidosis implies immune activation with

cytokine induction of MHC-II expression on mesangial cells and macrophages. Identifying the source of the inflammatory reaction may help in directing treatment and delaying or arresting progression to glomerulosclerosis.

#### **N-04: INTRATUMORAL EXPRESSION OF PROSTAGLANDIN EP4 RECEPTOR GENE PTGER4 IN MULTIPLE CANINE MALIGNANCIES**

Austin Viall, Rachel Phillips, Jesse Hostetter, Chad Johannes, Margaret Musser

**Background:** Chronic inflammation is a well-recognized pro-oncogenic factor and is associated with development or progression of many cancers. Cyclooxygenase products, especially prostaglandins, are major signal mediators of inflammation. Prostaglandin E2 drives a number of pro-tumor processes, such as cellular proliferation and angiogenesis, through interaction with four E2 specific receptors. EP4 receptor (EP4R) is expressed in multiple human cancers and is associated with tumor development; presence of EP4R is a poor prognostic indicator for some cancers. The expression of EP4R has been minimally evaluated in canine tumors, yet could serve as a novel therapeutic target.

**Objective:** Our study aimed to characterize the EP4R mRNA expression in canine osteosarcoma (OSA), transitional cell carcinoma (TCC), anal sac apocrine gland adenocarcinoma (ASAGACA), and squamous cell carcinoma (SCC).

**Methods:** RNA *in situ* hybridization (RNAscope) was used to assess EP4R mRNA transcript expression in 10 formalin-fixed samples of OSA, TCC, ASAGACA, and SCC. RNAscope signal was quantified with an image analysis system (HALO). Data was expressed as copy number for EP4R, with comparison to the housekeeping target (B-actin, positive control gene).

**Results:** In 10/10 tumor samples each of OSA, TCC, ASAGACA, and SCC, strong universal positive expression of EP4R was identified. These results confirm the mRNA expression of canine EP4R in all tumor types evaluated.

**Conclusions:** EP4R mRNA expression was identified in all canine tumor types evaluated. Our results indicate assessment of EP4R protein expression in these tumors is strongly warranted and that therapeutic blockade of EP4R with a specific antagonist may be a novel treatment.

#### **N-05: NORTH AMERICAN RIVER OTTER (LONTRA CANADENSIS) MORTALITY SURVEILLANCE, EAST CENTRAL FLORIDA, 2017-2018**

David Rotstein, Megan Stolen, Constance Silbernagel

**Background:** North American river otters (*Lontra canadensis*) are ubiquitous in Florida. In the Indian River Lagoon (IRL) watershed, otters are one of two top mammalian predators. Assessment of pathologic findings on a population level has not been done.

**Objectives:** To describe the pathologic findings in roadkill otters from the IRL.

**Methods:** From January 2016 to March 2018, 43 dead otters were collected. Necropsies were performed with sampling for histopathology.

**Results:** Males and females were nearly equally distributed. Juvenile and adult age classes were sampled. Gross and histologic evidence of vehicular blunt force trauma involving multiple organs, including ruptured, fractures, proptosis, and soft tissue hemorrhage was observed in nearly all cases (21/22). Non-traumatic lesions included eosinophilic and granulomatous pneumonia (4/22), lymphadenitis (4/22), thymic involution (5/22), intrasarcoplasmic sarcocysts (8/22), adrenalitis (2/22), hepatitis (3/22), subcutaneous Dracunculiasis and/or cestodiasis (4/22), and hepatic nodular hyperplasia (2/22).

**Conclusions:** Anthropogenic trauma was the primary cause of death in river otters this study. Incidental lesions were present in a few animals, and systemic infectious agents or significant organ-specific changes were not observed.

#### **N-06: USE OF GENETIC AND EPIGENETIC TOOLS TO DISCOVER MARKERS FOR HOST RESILIENCE TO OVINE PROGRESSIVE PNEUMONIA VIRUS IN SHEEP**

Alisha Massa, Michelle Mousel, Brenda Murdoch, Donald Knowles, J Taylor, Stephen White

**Background:** Genetic selection has been successful in sheep to combat important infectious diseases including scrapie and footrot. Ovine progressive pneumonia virus (OPPV) is a retrovirus, genus *Lentivirus*, closely related to human immunodeficiency virus. Previously, we discovered a genetic marker associated with 50% decreased OPPV in the blood (proviral load). Proviral load correlates with histological severity of pneumonia, arthritis, and mastitis. Therefore, this marker predicts resilience.

**Objective:** Our objective was to refine the genetic marker near four zinc finger genes to improve usefulness as a genetic test for producers and to elucidate possible mechanisms of host resilience.

**Methods:** Mutations that create amino acid substitutions in these four genes were identified, genotyped by polymerase chain reaction and restriction fragment length polymorphism, and tested for association with the resilient phenotype ( $p < 0.05$ ). Chromatin immunoprecipitation with high throughput sequencing (ChIP-seq) was completed on sheep alveolar macrophages to identify enhancers, genome wide, since 93% of causal genetic mutations that explain disease phenotypes in humans are predicted to lie outside of genes within regulatory elements.

**Results:** No mutations within genes were significantly associated with the phenotype. We have refined the location of the genetic marker to a region outside of any gene that is significantly associated with the resilient phenotype. The marker is within or very near an active enhancer upstream of *ZNF389*.

**Conclusions:** Our current model is that this OPPV resilient phenotype is explained by mutations in the host regulatory elements that alter gene expression of zinc finger transcription factors in sheep thereby inhibiting the virus.

#### **N-07: EXPRESSION OF OLIGODENDROCYTE PRECURSOR CELL MARKERS IN CANINE OLIGODENDROGLIOMAS**

Takuya Kishimoto, Kazuyuki Uchida, Atigan Thongtharb, Tokuhiko Shibato, James Chambers, Kazumi Nibe, Yumiko Kagawa, Hiroyuki Nakayama

**Background:** Oligodendroglioma is a common brain tumor in dogs, particularly brachycephalic breeds. Oligodendrocyte precursor cells (OPCs) are suspected to be a possible origin of oligodendroglioma, although it has not been studied in the dog.

**Methods:** In this study, 27 cases of canine brain oligodendroglioma were histologically and immunohistochemically examined.

**Results:** The most commonly affected breed was French Bulldog (19/27, 70%). Seizure was the predominant clinical sign (17/25, 68%). The tumors were located mainly in the cerebrum, particularly in the frontal lobe (10/27, 37%). All cases were diagnosed as anaplastic oligodendroglioma (AO) and had common histologic features characterized by the proliferation of round to polygonal cells with pronounced atypia and conspicuous mitoses (average, 10.7/10 high-power fields). Honeycomb pattern (5/27, 19%), myxoid matrix (10/27, 37%), cyst formation (6/27, 22%), necrosis (19/27, 70%), pseudopalisading (5/27, 18.5%), glomeruloid vessels (16/27, 59%), and microcalcification (5/27, 19%) were other histopathologic features of these tumors. Immunohistochemically, the tumor cells were positive for Olig2 in all cases and for other markers of OPCs in most cases, including SOX10 (24/27, 89%), platelet-derived growth factor receptor $\alpha$  (24/27, 89%), and NG2 (23/27, 85%). Tumors also consisted of heterogeneous cell populations that were positive for nestin (13/27, 48%), glial fibrillary acidic protein (5/27, 19%), doublecortin (22/27, 82%), and  $\beta$ III-tubulin (15/27, 56%). Moreover, established AO cell line from necropsied cases retained expression of OPC markers and exhibited multipotent characteristics in a serum culture condition.

**Conclusions:** The findings suggest that transformed multipotent OPCs may be a potential origin of canine AO.

#### **N-08: PATHOLOGY OF CETACEAN MORBILLIVIRUS AND COMORBIDITIES IN GUIANA DOLPHINS DURING 2017-2018 EPIDEMICS (BRAZIL)**

Kátia Groch, Josué Díaz-Delgado, Elitieri Santos-Neto, Joana Ikeda, Rafael Carvalho, Raissa Oliveira, Emi Guari, Eduardo Ferreira-Machado, Carlos Sacristán, Lara Keid, Rodrigo Soares, Gláucia Sousa, Daniella Oliveira, Alexandre Azevedo, José Lailson-Brito, José Catão-Dias

**Background:** Cetacean morbillivirus (CeMV; Paramyxoviridae) is a re-emergent pathogen associated with severe epidemic and endemic fatalities in cetaceans worldwide. We recently reported preliminary molecular and pathologic results attesting to the major role of Guiana dolphin strain (GD)-CeMV on an unusual mass-mortality

event that claimed more than 250 Guiana dolphins (*Sotalia guianensis*) in Rio de Janeiro (November 2017 to February 2018).

**Objectives:** To provide larger-scale molecular, pathologic and immunohistochemical investigations on the Guiana dolphin fatalities with major emphasis on CeMV and comorbidities.

**Methods:** We performed RT-PCR (n=90) analysis, and detailed gross, histopathologic and immunohistochemical (n=32) examinations.

**Results:** Tissue samples from 82/90 (91%) animals were positive for GD-CeMV by RT-PCR. Detailed post mortem examination was performed in 32/82 (39.0%) CeMV-positive animals. Most prevalent gross findings were: lack of ingesta, verminous pneumonia by *Halocercus brasiliensis*, pulmonary edema, ascites, icterus, hepatic lipidosis, and gastrointestinal parasitism. Microscopically, consistent GD-CeMV-associated lesions included moderate to severe bronchointerstitial pneumonia with type II pneumocyte hyperplasia, syncytia and viral inclusion bodies, typically overlapped by chronic *H. brasiliensis* parasitosis; and multicentric lymphoid depletion with syncytia, followed by eosinophilic lymphadenitis by *H. brasiliensis*. There was widespread lymphohistiocytic and epithelial CeMV-antigen distribution, but neuronal/neuroglial labeling was rare. Most animals had acute/subacute systemic presentation based on current knowledge on cetacean morbillivirosis. Severe, often fatal comorbidities included: *Brucella* sp. (1/68; 1.4%); disseminated toxoplasmosis (3/32; 9.37%), disseminated mycosis (2/32; 6.25%) and multisystemic ciliate protozoosis (2/32; 6.25%).

**Conclusions:** These results further contribute to delineate the pathologic and molecular characteristics of the first CeMV epidemics in South-American cetaceans.

#### **N-09: SERUM CYTOKINE AND GROWTH FACTOR PROFILING OF HEALTHY DOGS AND DOGS WITH OSTEOSARCOMA VIA MULTIPLEX ANALYSIS**

Carolina Allende, Tracy Jamison, Berta Higgins, Jennifer Johns

**Background:** Serum or plasma analysis for biomarker use in companion animals with cancer is becoming a topic of increasing interest. Minimal information is published on circulating cytokine and growth factor evaluation in dogs with naturally occurring osteosarcoma. Assessment of circulating biomarkers in dogs with osteosarcoma may have clinical veterinary importance.

**Objective:** To compare serum cytokine and growth factor levels between healthy dogs and dogs with spontaneous osteosarcoma.

**Methods:** Serum was obtained from 14 dogs with naturally occurring osteosarcoma and from 18 healthy dogs that were roughly age- and weight-matched to the osteosarcoma patients. Multiplex analysis was used to quantify serum levels of 12 analytes including tumor necrosis factor-alpha (TNF-alpha), monocyte chemoattractant protein 1

(MCP1/CCL2), vascular endothelial growth factor (VEGF) and transforming growth factor beta 1 (TGF-beta 1).

**Results:** Significant increases in serum MCP1 and TNF-alpha levels were found in dogs with osteosarcoma compared to healthy dogs. Several other analytes including TGF-beta 1 were found to have a non-significant trend toward increase in dogs with osteosarcoma compared to healthy dogs. All except one of the dogs with osteosarcoma had current NSAID administration at the time of diagnosis and serum sampling.

**Conclusion:** Serum cytokine profiles differ between dogs with osteosarcoma and healthy dogs, reflecting immune response alterations. Future work will focus on using serum multiplex analysis as a potential prognostic tool in canine osteosarcoma, and on determining how local and systemic host immune responses may influence tumor progression and metastasis.

#### **N-10: ANALYSIS OF EPHA3 EXPRESSION IN CANINE BRAIN NEOPLASIA**

Hannah Diebold, Emily Jones, Adam Whitelock, Hoi Ching Leung, Simon Puttick, Rachel Allavena

**Background:** Brain cancer has a poor prognosis with limited treatment and diagnostic options in canine patients. Ephrin receptor A3 (EphA3) controls a range of functions in developing central nervous tissue including cell attachment, cell migration, and axonal guidance, and is highly expressed in embryological brain, spinal cord, kidney, heart, and lungs. Expression decreases during maturation but may become upregulated during oncogenesis with overexpression linked to more aggressive neoplasms in people.

**Objective:** To analyse the expression of EphA3 in a range of canine brain neoplasms and normal canine tissue for the potential development of novel theranostic agents and use in translational medicine.

**Methods:** Immunohistochemical analysis was performed on 60 formalin-fixed, paraffin embedded canine neoplasms (17 astrocytomas, 5 glioblastomas, 15 oligodendrogliomas, and 23 meningiomas) from the University of Queensland School of Veterinary Science pathology archives using a rabbit polyclonal EphA3 antibody (bs-7032R, 1:100 dilution). Canine kidney was selected as a control for its known positive expression of EphA3. Samples were graded using light microscopy based on intensity of staining (0-3) and percentage of cells stained (0%-100%).

**Results:** Normal canine brain tissue showed strong axonal and ependymal EphA3 staining with variable minimal to moderate staining of neurons and endothelium. Neuropil showed minimal, ubiquitous staining, while astrocytes and oligodendrocytes remained unstained. Brain neoplasms were largely negative to minimally stained.

**Conclusion:** The highly positive expression of EphA3 in normal canine brain tissue and its downregulation in brain neoplasms indicates EphA3 is most likely inappropriate for use as a theranostic target in canine patients.

## **N-11: METASTASIS OF AN EQUINE OCULAR SQUAMOUS CELL CARCINOMA TO THE GUTTURAL POUCH**

José Ramos, Margaret Miller, Mario Sola, Wendy Townsend, Kaitlin Milenicki, Gillian Haanen

**Background:** A 15-year-old Clydesdale mare developed anorexia and weight loss. Endoscopic evaluation revealed firm, pale nodules in the right guttural pouch. Seven years earlier, a right third eyelid mass had been excised without histologic examination. However, when the horse developed corneal plaques in the medial canthus a year later, the right orbit was exenterated. The histologic diagnosis was corneal and conjunctival squamous cell carcinoma (SCC).

**Objective:** Pathology of a metastatic squamous cell carcinoma in the guttural pouch.

**Methods:** Biopsy tissue from the guttural pouch was evaluated histologically with a diagnosis of SCC; the mare was euthanized and submitted for autopsy.

**Results:** A 5 cm x 7 cm firm, tan, multinodular mass was deep to mucosa along the medial aspect of the right guttural pouch with smaller nodules along the lateral aspect. Regional lymph nodes were enlarged with caseation. Skeletal muscle lateral to the right stylohyoid bone was pale and firm. Histologically, all grossly affected tissues plus an 8-mm pulmonary nodule and a tracheobronchial lymph node contained SCC with emboli in lymphatic vessels. The SCC consisted of cords and clusters of atypical keratinocytes with 25 mitotic figures in 2.37 mm<sup>2</sup> (ten 400X fields), keratin pearl formation, and scant collagenous stroma.

**Conclusions:** The history and the lack of continuity between mucosal epithelium and the tumor make metastatic rather than primary the more likely origin of the guttural pouch SCC. Equine guttural pouch neoplasms are rare, but SCC is the most commonly reported. Other guttural pouch neoplasms include hemangioma, fibroma and melanoma.

## **N-12: CHARACTERIZATION OF ENTEROADHERENT BACTERIA WITH ZOONOTIC POTENTIAL IN SMALL INTESTINAL BIOPSY SPECIMENS OBTAINED FROM SENIOR CATS WITH DIARRHEA AND VOMITING**

Elena Demeter, Eunjun Choi, Kenny Simpson, Gerald Duhamel

**Background:** Gastrointestinal diseases including inflammatory bowel disease and alimentary lymphoma are important considerations in geriatric cats. Intestinal infection of young kittens with attaching-effacing *Escherichia coli* (AEEC) and *Enterococcus hirae* have been described previously.

**Objective:** Characterize enteroadherent bacteria in small intestinal biopsy specimens obtained from five senior cats (mean 12.8-year-old, range 8-13) with diarrhea and vomiting.

**Methods:** Gastrointestinal biopsies obtained from 5 senior cats with enteroadherent bacteria along the apical membrane of villous enterocytes by routine histopathological

examination were characterized by: (i) Gram stain (n=5), (ii) fluorescent *in situ* hybridization (FISH) with a universal eubacterial and probes specific for *E. coli/Shigella* and *Enterococcus* spp. (n=5), (iii) transmission electron microscopy (TEM; n=2), and (iv) bacteriological culture (n=2) and whole-genome sequencing (n=1).

**Results:** Moderate to severe enteritis, together with infection by Gram negative (n=3), Gram positive (n=1), or both (n=1) were confirmed by FISH. AEEC was confirmed in 3 cases with Gram negative bacterial infection, *Enterococcus* spp. in one case with Gram positive bacterial infection, and a combination of both AEEC and *Enterococcus* spp. in one case. Intimate adhesion of coccobacilli and coccoid bacteria to the apical cell membrane of villous enterocytes was confirmed by TEM of small intestinal specimens taken from one cat with AEEC and one cat with *Enterococcus* spp., respectively. Bacteriological culture of prospective stool samples taken from two affected cats confirmed infection with *E. coli* or *Enterococcus hirae*, respectively.

**Conclusions:** Enteroadherent bacteria contribute to enteritis and should be considered as important differentials in aged cats with gastrointestinal signs.

### **N-13: EXTRAINTESTINAL ESCHERICHIA COLI CAUSES NECROHEMORRHAGIC PNEUMONIA IN MULTIPLE RESEARCH DOGS**

Michelle Magagna, April George, Caitlyn Carter, Charissa Dean, Keith Nelson

**Background:** Pneumonia is an uncommon but serious cause of morbidity in beagle research dogs. It is often associated with pulmonary misdosing or potential test-article effects, rather than infectious etiologies. Extraintestinal pathogenic *Escherichia coli*, particularly strains expressing cytotoxic necrotizing factor (CNF) 1 and 2 virulence factors, is a rarely reported cause of acute, fatal necrohemorrhagic pneumonia in dogs.

**Objective:** The purpose of this study was to review cases of acute necrohemorrhagic pneumonia in beagle dogs at our facility between 2011-2017.

**Methods:** Clinical presentation, macroscopic and microscopic findings, and laboratory testing results in 13 dogs (8 male, 5 female) with suspected *E. coli* pneumonia were assessed. Almost all dogs died or were euthanized *in extremis* after a short course of clinical symptoms or following receipt from suppliers.

**Results:** Clinical symptoms were typically fulminant, consisting of lethargy, dyspnea, and hemorrhage. In all dogs, affected lung lobes were variably discolored dark red and consolidated. Few dogs had pleural effusion or multisystemic hemorrhage. Pulmonary histologic lesions included alveolar necrosis, hemorrhage, edema, fibrin deposition, acute inflammation, and intralesional colonies of bacterial bacilli. Lungs were cultured from nine dogs and *E. coli* isolated; for 7 of these dogs, virulence factor PCR was performed and identified CNF-1, with no other factors identified.

**Conclusions:** Extraintestinal *E. coli* is an emerging, important cause of acute fatal necrohemorrhagic pneumonia in purpose-bred beagle research dogs and may often be associated with a recent history of transport.

#### **N-15: COMPARATIVE HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL INVESTIGATIONS OF CETACEAN MORBILLIVIRUS BETWEEN MEDITERRANEAN AND NORTHEAST-CENTRAL AND SOUTHWESTERN ATLANTIC CETACEANS: PRELIMINARY RESULTS**

Josue Diaz-Delgado, Katia Groch, Eva Sierra, Simona Sacchini, Daniele Zucca, Oscar Quesada-Canales, Manuel Arbelo, Antonio Fernandez, Rodrigo Ressio, Cristina Kanamura, Elitieri Santos Neto, Joana Ikeda, Rafael de Carvalho, Alexandre Azevedo, José Lailson-Brito Jr, Cíntia Favero, Cinzia Centelleghé, Sandro Mazzariol, Ludovica Di Renzo, Gabriella Di Francesco, Giovanni Di Guardo, Jose-Luiz Catão-Dias

**Background:** Cetacean morbillivirus (CeMV) is the most impactful natural cause of morbidity and mortality in cetaceans worldwide. However, the pathogenetic intricacies are not fully resolved with many questions remaining unanswered, particularly concerning pulmonary and central nervous system (CNS) disease and the mechanisms of immunosuppression.

**Objective:** To compare histopathologic and immunohistochemical features between CeMV-PCR-positive Guiana dolphins (GDs; *Sotalia guianensis*) from Brazil, and striped dolphins (SDs; *Stenella coeruleoalba*) and bottlenose dolphins (BDs; *Tursiops truncatus*) from the Canary Islands and Italy.

**Methods:** Eleven GDs, 13 SDs and three BDs were selected by defined criteria and were subjected to histopathologic and immunohistochemical analyses. Major emphasis was placed on the CNS, lymphoid system and lung.

**Results:** CeMV infections showed remarkable neurotropism characterized by meningoencephalitis in SDs and BDs, while this was a rare feature in GDs. GDs tended to have more severe lung lesions, typically bronchointerstitial pneumonia. The lymphoid system was affected in all three species, with consistent lymphoid depletion. Overall, there was widespread lymphohistiocytic, epithelial and neuronal/neuroglial CeMV-antigen detection with some individual, host and strain differences. Neuroanatomical distribution of lesions in Canarian dolphins revealed consistent involvement of the cerebral cortex, thalamus and cerebellum, followed by brainstem and spinal cord. Preliminary immunohistochemical results indicate CNS-CeMV inflammation involves CD3-, IgG-, PAX5-, lysozyme- and caspase 3-positive cells; same immunomarkers evidenced CeMV-associated alterations in lymphoid organs and lung. Coinfections were common.

**Conclusions:** These results contribute to understanding convergences and divergences of CeMV infections between CeMV strains, hosts, geographic locations and set the basis for future immunopathological comparative investigations.

#### **N-16: HISTOLOGIC CHARACTERIZATION OF THE MAJOR DUODENAL PAPILLA OF CATS AND CORRELATION TO PRESENCE OF CHOLANGITIS, PANCREATITIS, AND ENTERITIS**

Megan Schreeg, John Cullen, Jody Gookin

**Background:** The clinical syndrome of “triaditis” is commonly diagnosed in cats, but the pathogenesis is poorly understood. The prevailing theory is that conjoining of the major pancreatic and common bile ducts prior to emptying at the major duodenal papilla predisposes cats to concurrent cholangitis, pancreatitis, and enteritis. However, the histomorphology and presence of pathology at this anatomic site has not been assessed in cats with or without triaditis.

**Objective:** The aim of this study was to determine if there is any association between major duodenal papilla duct anatomy or pathology and the presence of cholangitis, pancreatitis, or enteritis in cats.

**Methods:** Samples of major duodenal papilla were collected opportunistically for routine histologic assessment from 46 cats presented for postmortem examination. Concurrent samples of liver, pancreas, jejunum, and ileum were obtained from 35 of these cats.

**Results:** Separate pancreatic and common bile ducts were identified at the major duodenal papilla in 20% (9/46) of cats, with the remaining demonstrating a complex ductular network. Lymphoid aggregates/follicles were present at the major duodenal papilla of 47% (22/46) of cats, and inflammation was identified in 28% (10/35). Cholangitis (31%, 11/35), pancreatitis (57%, 20/35), and enteritis (37%, 13/35) were common, but none of these were associated with inflammation at the major duodenal papilla. Triaditis was less common (11%, 4/35), but 3/4 of these cats had concurrent inflammation at the major duodenal papilla.

**Conclusions:** These findings suggest that immune activation or inflammation of the major duodenal papilla may play a role in the pathogenesis of triaditis.

#### **N-17: HISTOLOGIC LESIONS IN CAPTIVE AMERICAN HORSESHOE CRABS (LIMULUS POLYPHEMUS)**

Elise LaDouceur, Lisa Mangus, Michael Garner, Andrew Cartoceti

**Background:** American horseshoe crabs (HSC) are arthropods that are commonly kept in aquaria and occasionally submitted to pathologists for histologic examination. A histologic review of diseases commonly encountered in captive HSC is not currently available. This review describes histologic findings in captive HSC to aid pathologists in evaluating this species.

**Methods:** Histologic tissue sections of HSC cases submitted to three diagnostic institutions were reviewed.

**Results:** Slides from 66 HSC were reviewed. Organs that were routinely evaluated included the carapace, hepatopancreas, book gills, digestive tract, coxal gland, circulatory system (including heart), nervous system (including brain), gonads, and eyes. Infectious diseases were commonly diagnosed, including infections by fungi, bacteria, and parasites. Bacterial and fungal infections were associated with robust inflammation, consisting of hemocytic infiltration and coagula formation. Fungal infection commonly caused hemocytic, erosive to ulcerative dermatitis and branchitis. Fungal culture occasionally identified *Fusarium* spp., though fungal morphology varied and

multiple fungal species may have been present. Bacterial infection was frequently with Gram negative bacteria, and commonly caused dermatitis and multifocal hepatopancreatitis. The most common parasite infection was metacercariae (presumed *Microphallus limuli*) in numerous connective tissues; these were associated with minimal hemocytic or no appreciable inflammation. Molting abnormalities were common, and were frequently associated with secondary carapacial infection. Decreased eosinophilic vacuoles were noted in the hepatopancreatic interstitial cells in some cases, which may indicate decreased nutritional status.

**Conclusion:** Detailed studies on the histopathology of HSC will aid pathologists in providing useful information to aquarists/veterinarians to improve management and care of these animals.

#### **N-18: INVESTIGATING MOLECULAR MARKERS USING TISSUE MICROARRAYS FOR THE PROGNOSIS OF CANINE MAST CELL TUMORS**

Britta Knight, Geoffrey Wood, Robert Foster, Brenda Coomber

**Background:** Mast cell tumors (MCTs) are the most common skin tumor of the dog, representing approximately 21% of all cutaneous tumours. Accurately predicting behavior is critical in directing patient therapy. It is especially important in canine MCTs as they range from benign to a fatal systemic disease. Grading is useful for prognosis, but it cannot predict the behavior of each MCT.

**Objectives:** We hypothesized that biomarker expression in tumor cells will correlate with patient outcome, which will help guide future therapy.

**Methods:** A tissue microarray (TMA) from dermal, subcutaneous, and metastatic canine MCTs was created. A TMA is an array-based technique that enables high-throughput expression profiling of large numbers of tissues or tumors. We performed immunohistochemistry (IHC) for 10 different proteins (Beclin-1, C-CBL, VEGFR2, PDGFR- $\beta$ , c-kit, mast cell tryptase, Ki-67, PCNA, vWF, CD31) on neoplastic and positive and negative control tissue. Each tumor was analyzed to derive an H-score, which is a function of the intensity of IHC staining and the percentage of immunopositive cells.

**Results:** The analysis showed a positive correlation between cytoplasmic Beclin-1 expression and expression of cytoplasmic KIT.

**Conclusions:** As cytoplasmic KIT immunopositivity correlates with increased rates of local recurrence and decreased survival, Beclin-1 may be a promising new molecular biomarker to improve prognosis and guide adjuvant therapy for canine MCTs.

#### **N-19: UTERINE LESIONS OF DOMESTIC RABBITS (ORYCTOLAGUS CUNICULUS) IN JAPAN: A RETROSPECTIVE COHORT STUDY**

Hirotaka Kondo, Kanako Settai, Hisashi Shibuya

**Background:** Uterine lesions are frequently diagnosed in domestic rabbits; however, retrospective studies are limited.

**Objective:** The purpose of this study was to clarify the variation of uterine lesions, age distribution, breed predilection, and related clinical signs in affected rabbits.

**Methods:** Cases included in the study were rabbits with a history of ovariohysterectomy due to possible uterine disease or for disease-prevention purposes, with subsequent histopathologic examination of removed uteri. Data comprised clinical and pathologic records of 1935 rabbits treated between 2009 and 2018 at 43 veterinary hospitals belonging to the Japanese Society of Exotic Pet Medicine (JSEPM) by a questionnaire through the JSEPM website.

**Results:** The most common rabbit breeds were mixed breed (604 cases, 31%), Netherland dwarf (523 cases, 27%), and Holland lop (289 cases, 14%). A total of 1338 neoplastic lesions and 982 non-neoplastic lesions were detected. Most cases showed two or more lesions. The most common neoplastic lesion was endometrial adenocarcinoma (1030 cases), followed by leiomyoma (162 cases), leiomyosarcoma (80 cases), and carcinosarcoma (37 cases). Cystic endometrial hyperplasia was the most common non-neoplastic lesion (786 cases). The clinical sign most frequently related to both neoplastic and non-neoplastic uterine lesions was hematuria, and 547 cases with endometrial adenocarcinomas showed hematuria. No clinical signs before ovariohysterectomy were seen in 141 cases, although some uterine lesions were identified on histopathologic examination.

**Conclusion:** These results support the recommendation for prevention by ovariohysterectomy before 2 years old as key to the management of uterine lesions, particularly to reduce the risk of endometrial adenocarcinoma.

## **N-20: COMPARISON OF EXPRESSION OF THE APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER ALONG THE INTESTINAL TRACT BETWEEN DOGS WITH AND WITHOUT CHRONIC ENTEROPATHY**

Paula Giaretta, Raquel Rech, Blake Guard, Amanda Blake, Anna Blick, Joerg Steiner, Jonathan Lidbury, Audrey Cook, Mohsen Hanifteh, Thomas Spillmann, Susanne Kilpinen, Pernilla Syrjä, Jan Suchodolski

**Background:** Fecal bile acid dysmetabolism has been reported in humans with inflammatory bowel disease and in dogs with chronic enteropathy (CE).

**Objective:** Because intestinal absorption of bile acids is mediated by the apical sodium-dependent bile acid transporter (ASBT), we aimed to characterize ASBT distribution along the intestinal tract (duodenum, jejunum, ileum, cecum, and colon) of control dogs. Then we compared ASBT expression in the ileum and colon to dogs with CE.

**Methods:** ASBT mRNA and protein expression was assessed using RNA in situ hybridization and immunohistochemistry, respectively. Fecal bile acid concentration was determined with gas chromatography–mass spectrometry. The fecal microbiota dysbiosis index was determined by a qPCR panel.

**Results:** ASBT mRNA expression was observed in the nucleus and cytoplasm of enterocytes in all analyzed intestinal segments, with the highest levels expressed in the

ileum. ASBT protein expression was restricted to the ileum, cecum, and colon with immunolabeling distributed on the apical membrane of enterocytes. Dogs with CE had significantly decreased expression of ASBT protein in the ileum that correlated with the histopathological scores. Additionally, dogs with CE had significantly increased percentage of primary BAs in their feces compared to controls. The fecal dysbiosis index was significantly higher in dogs with CE.

**Conclusions:** We concluded that ASBT protein expression in the ileum is downregulated in dogs with CE, which might be linked to the inflammatory process, intestinal dysbiosis, and fecal bile acid dysmetabolism.

## **N-21: NEUROPATHOLOGICAL STUDY OF INCLUSION BODY DISEASE OF JAPANESE BROWN CATTLE**

Ken-ichi Watanabe, Mitsue Yokoyama, Kazuyuki Uchida, James Chambers, Hiroyuki Nakayama, noriyuki Horiuchi, Hidefumi Furuoka, Takane Matsui, Andrew Miller, Yoshiyasu Kobayashi

**Background:** Inclusion body disease of Japanese brown cattle (IBD) is a fatal neurologic disease with hyperexcitability, profuse sweating, anorexia and fever. Histologically, eosinophilic cytoplasmic inclusion bodies are occasionally observed in large neurons of midbrain, pons and medulla. Although IBD was firstly reported in 1988, the pathogenesis of IBD was not elucidated.

**Research objects:** To characterize immunohistochemical and ultrastructural features of IBD.

**Methods:** Eleven cases of IBD were used (9 females and 2 males with ages varying from 10 months to 4 years). Immunohistochemistry were performed on formalin-fixed paraffin-embedded tissues. Midbrain of 3 cases were also examined by transmission electron microscopy.

**Results:** Eosinophilic cytoplasmic inclusions, approximately 50µm in diameter, were observed in large neurons of reticular formation, red nucleus, motor nerve nuclei (oculomotor nucleus, glossopharyngeal nucleus, facial nerve nucleus), dorsal horn and ventral horn. Ultrastructurally, the inclusions had an electron-dense core, and consisted of degenerated mitochondria and loose thin filamentous bundles. The inclusions are immunopositive for ubiquitin and cytochrome C. Immunohistochemistry for common autophagy markers such as LC3 and p62 revealed granular to diffuse cytoplasmic immunoreactivity in neurons with/without inclusions. Around the neurons with inclusions, immunohistochemistry for superoxide dismutase 1 (SOD-1) revealed granular immunoreactivity in neuropile. Moreover, in the severest case with numerous inclusions in widespread areas, the inclusions were immunopositive for SOD-1.

**Conclusions:** IBD was presumed to be a motor neuron disease associated with mitochondrial damage. The immunoreactivity of LC3 and p62 might imply the involvement of autophagy mechanisms in the pathogenesis of IBD.

## **N-22: CARDIAC MESOTHELIAL HYPERPLASIA IN CATTLE**

Dayna Goldsmith, John Adaska

**Background:** Epicardial proliferations of mesothelial cells and fibrovascular tissue, known as milk spots or soldier's plaques, are a well-recognized incidental lesion of aging in humans. Recognition of this entity is important to prevent misdiagnosis as pericardial disease. These lesions are believed to arise from chronic friction between the epicardium and pericardium. Similar proliferations have been described in mice with sternal dislocations, and in laboratory beagles as an incidental finding. Cardiac mesothelial hyperplasia has been observed in adult cattle and a previous description of three cases correlated the lesion with infection with bovine leukosis virus (BLV).

**Objective:** Characterize and determine the prevalence of cardiac mesothelial hyperplasia in cattle and evaluate the potential association with BLV.

**Methods:** Hearts from routine diagnostic cases (15) and from a local rendering facility (53) were collected and examined for mesothelial hyperplasia. When available, blood and/or spleen samples from the same animals were tested for BLV by ELISA. Basic morphometric measurements of the hearts were also collected and histologic examination of several proliferative epicardial lesions was performed.

**Results:** Of the 68 hearts examined, only two lacked grossly discernible mesothelial hyperplasia. Of the 68 animals sampled, 15 were positive for BLV, including one without proliferative epicardial lesions. The most frequent location for mesothelial hyperplasia was around the great vessels, especially the pulmonary trunk, and there was variation in gross morphology.

**Conclusions:** Mesothelial hyperplasia is a common incidental finding in adult cattle and does not appear to be associated with BLV infection.

## **N-23: THE USE OF HISTOPATHOLOGICAL STAINS TO DOCUMENT STRETCHED COLLAGEN IN THE TISSUE LESIONS OF EQUINE NAVICULAR DISEASE**

Elizabeth Uhl, Uriel Blas-Machado, Shannon Kirejczyk, Michelle Osborn

**Background:** Navicular disease is a common form of degenerative joint disease in horses that currently can only be managed but not effectively treated. In cases of navicular disease, lesions are found throughout the navicular apparatus, which is a functional enthesis organ highly adapted to dissipate forces away from the osteotendinous junction between the deep digital flexor tendon and the distal phalanx. Given this function, mechanical overloading is considered to be a primary cause of navicular disease; however lesions due to mechanical stress are difficult to document pathologically.

**Objective & Methods:** For this study, we used the paradoxical property of Masson's trichrome and picrosirius red staining to document the presence of stretched collagen in the navicular enthesis organs of horses with and without navicular disease.

**Results:** Evidence of stretched collagen was extensive in the navicular cases. One had a large, locally extensive area of stretched collagen that correlated with the deep flexor tendon lesions identified by MRI. Degeneration of the navicular fibrocartilage and bone was also characterized by evidence of marked collagen tension, as was a locally extensive area in the underlying digital cushion. Lesions ranging from disruption and loss of collagen fibers to deposits of cartilage and bone were also documented in the impar entheses of the navicular cases.

**Conclusions:** These findings confirm the primary mechanical pathogenesis of navicular disease and indicate the utility of using Masson's trichrome and picrosirius red staining to document mechanical stress in tissue.

#### **N-24: ERYSIPELAS BACTEREMIA IN A SERIES OF ZOO BIRDS**

Joseph Malatos, Patrick Mitchell, Laura Goodman, Charlotte Hollinger

**Background:** *Erysipelothrix rhusiopathiae* infections are reported sporadically across taxa, with only rare reports in aquatic birds. From 2012-2018, six cases of acutely fatal *Erysipelothrix* bacteremia occurred in four different avian species in a zoological collection.

**Objective:** The objective of this case series was to review clinical and pathologic findings for consistent diagnostic features across six cases of Erysipelas in captive non-domestic birds.

**Methods:** Medical and pathology records were reviewed, and post-mortem tissue imprint cytology preparations (spleen, liver, and lung), routine histology of all major organs, and Gram-stained sections were assessed microscopically. Bacterial cultures were performed on fresh or frozen tissue and whole genome sequencing was used to assess culture isolates.

**Results:** The six cases consisted of three Inca terns, one pied avocet, one giant wood rail, and one red-knobbed imperial pigeon; all were male. Four cases were 2-6 months old (range 2 months to 11 years). All cases were found dead with no premonitory signs. Repeatable gross findings included mild to moderate splenomegaly (4/6) and wet lungs (3/6). Cytology of spleen (6/6), liver (5/5) and lung (5/5) showed prominent leukocyte vacuolation and intracellular mildly pleomorphic bacilli. In all cases, histology showed circulating leukocytosis with intracellular bacteria and bacterial emboli. Bacteria were Gram positive (5/5) and bacterial culture identified *E. rhusiopathiae* (6/6) with variable genetic relatedness.

**Conclusions:** *Erysipelothrix rhusiopathiae* is a cause of acute fatal bacteremia in multiple avian species. Postmortem cytology aids in rapid identification of bacteremia. Young birds and environmentally or dietarily-exposed birds may be at increased risk.

## **N-25: EVALUATING A FUNGAL METALLOPROTEASE IN ASSOCIATION WITH CONIDIAL ADHERENCE TO EXTRACELLULAR MATRIX IN BAT WHITE-NOSE SYNDROME**

Piyaporn Eiamcharoen, Michael Keel

**Background:** *Pseudogymnoascus destructans* (Pd) is the etiological agent of white-nose syndrome (WNS) in hibernating bats. This emerging disease has caused dramatic mortality, leading to a decline in multiple bat species. Characteristic lesions are severe erosion and ulceration of the skin epidermis, especially on the patagium, muzzle and ears. Fungal adherence to host cells and extracellular matrix (ECM) provides the critical step to initiate the infection. Recent Pd transcriptome studies reveal that a Pd metalloprotease, which is similar to *Aspergillus fumigatus* major allergen (Asp f 2) could be involved in this process.

**Objective:** Our objective was to characterize the adherence process of Pd conidia to ECM and evaluate the role of Asp f 2 homolog as the possible underlying mechanism of Pd adherence.

**Methods:** The adherence assay was performed on 96-well plates of either immobilized laminin or fibronectin at different concentrations (1, 10, 50, 100 and 500mg/ml). Pd conidia was added at the density of  $10^5$  conidia per well and incubated at 12°C for 1, 3, 6, 12 or 24 hours. The number of conidia remaining adherent to the plate after washing were observed by a phase-contrast microscopy.

**Results:** Pd conidia adhered to laminin and fibronectin in the dose and time-dependent and saturable manner.

**Conclusions:** The adherence of Pd conidia to ECM demonstrates that Pd conidia could bind to ECM in vitro. This explanation could be an underlying mechanism of Pd infection of bat WNS.

## **N-26: INTRAOCULAR HISTIOCYTIC SARCOMA IN DOMESTIC CATS**

Megan Climans, Gillian Shaw, Richard Dubielzig, Leandro Teixeira

**Background:** Feline histiocytic sarcomas (HS) are rare malignant neoplasms, typically of interstitial dendritic cell origin that reportedly occur most often in spleen/skin or as disseminated disease. This is the first case series describing intraocular HS in cats.

**Objective:** To characterize the morphologic features and biologic behavior of intraocular HS in domestic cats.

**Methods:** Cases of feline HS from the COPLOW database were reviewed. Typical cases with positive IHC reaction to CD204 were included. Signalment, clinicomorphologic features, and follow-up information were evaluated.

**Results:** Eleven cases (2003-2018) met inclusion criteria. Median age at enucleation was 10 (4-17 years). No sex/breed predilection was found. Uveitis, glaucoma, and corneal ulcers were the most common clinical secondary ocular lesions. A neoplasm

was identified prior to enucleation in 6/11 cases. Neoplasia was localized in the anterior uvea in 9/11 cases, usually forming a well-delineated, pale tan or white mass grossly but a poorly delineated mass histologically. Histologic features were consistent with those described for feline HS elsewhere. Minimum survival time (time between enucleation and last-known-contact), ranged from 2-46 months. 6/11 cases were alive and 3/11 dead at last known contact. 2/11 were lost to follow-up. No cases had signs of systemic disease at enucleation nor signs of recurrence or metastasis at last follow-up.

**Conclusions:** Feline ocular HS are rare, but remain a differential for uveal round cell tumors. Minimum survival-time in cats was higher than survival-time of dogs with ocular HS. Lack of systemic disease in most of our cases might suggest a primary ocular HS.

#### **N-27: REVISITING THE CORRELATION OF C-KIT MUTATION STATUS AND TREATMENT DECISIONS IN CANINE MAST CELL TUMORS**

Sabine Hammer, Amina Paquay, Andrea Fuchs-Baumgartinger, Nicole Luckschander-Zeller, Armin Saalmüller, Ilse Schwendenwein, Barbara Rütgen

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

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

**Methods:** In 35 MCT dogs, the *c-Kit* exons 8, 9, 11, 13 and 17 were investigated. Therefore, total genomic DNA was isolated from remnant diagnostic material, PCR amplified and the obtained sequences were compared to healthy and malignant reference material.

**Results:** In total, no internal tandem duplications were detected but nine patients showed point mutations in exon 11. Among these, eight patients showed the same silent mutation, which has not been detected in the reference material. In one patient, the high-grade (Kiupel)/grade III (Patnaik) tumor stage did correlate with an amino acid exchange V563D (T>A<sup>1688</sup>). This driving mutation in human gastrointestinal stromal tumors could therefore also be an activating mutation in canine mast cell tumors.

**Conclusions:** For *c-Kit* mutation analysis, a time- and cost-efficient work routine was established. The absence of ITDs in exon 8 and 11 suggest that the use of the *c-Kit* mutation status alone is not sufficient to make treatment decisions.

#### **N-28: A 35-YEAR RETROSPECTIVE REVIEW OF DIAGNOSTIC DATA FROM NATIVE BATS (ORDER: CHIROPTERA) IN THE SOUTHEASTERN USA**

Shawn Mehrpad, Kevin Niedringhaus, Mark Ruder, Charlie Bahnson, Martha Dalton, Nicole Nemeth

**Background:** The order Chiroptera encompasses bat species occupying diverse niches and with key roles in agriculture, ecotourism, and zoonotic disease transmission. Bats are also subjected to anthropogenic pressures such as trauma (e.g., structure collisions, feral/domestic cat-induced) and disease spread (e.g., white nose syndrome; WNS).

**Objective:** We assessed for causes and contributors to morbidity and mortality as well as taxonomic, demographic, temporal, or geographic patterns among all bat submissions to the Southeastern Cooperative Wildlife Disease Study from 1983-2018.

**Methods:** A total of 689 bat submissions were reviewed (some submissions comprised >1 bats) that originated from 22 states concentrated in the southeastern USA. Data included: age, sex, location, date, clinical history, gross, histopathology, ancillary test results and final diagnoses. Causes of mortality were categorized as infectious (e.g., bacterial, viral, fungal), traumatic, toxicoses and physiologic stress.

**Results:** The cause of death was determined in 292 submissions, with trauma the most common (51.4%) followed by mycosis (11.0%). Of 387 samples tested for *Pseudogymnoascus destructans* (causative agent of WNS), 7.8% tested positive. Among 40 bats tested for rabies, 3.9 % tested positive, representing 80.0% of the cases with viral causes of mortality. Toxicoses (9.9%) were not diagnosed after 1991. Most submissions of known age (n=495) were adults (74.5%), followed by juveniles (19.6%) and pups/neonates (5.9%). Most of 689 submissions were from North Carolina (16.2%), Georgia (15.5%), and South Carolina (12.3%).

**Conclusion:** These data provide a rare opportunity to examine a long-term dataset of bat mortalities for aid in management strategy development and future research directions.

## **N-29: CAUSES OF NEONATAL KID MORTALITY IN ONTARIO DAIRY GOATS**

Emily Rätsep, Cathy Bauman, Jeffrey Wichtel, Julia Kim, Paula Menzies, Brandon Plattner, Robert Foster

**Background:** Ontario dairy goat producers identified neonatal mortality as a major economic and welfare issue (estimating 20-30% of kids). The actual mortality rate and causes is unknown and published data is scant. This study will benefit the dairy goat industry in Ontario, and internationally.

**Objective:** Provide rate and causes of mortality by age in dairy goat kids in Ontario.

**Methods:** A questionnaire mailed to 125 randomly sampled Ontario dairy goat producers identified 30 farms (stratified by region and lactating herd size) selected for a cohort study over 12-months. Postmortem examinations were performed on all kids that died between 0 and 120 days of age (stillbirths excluded). Of these, 10% had ancillary testing.

**Results:** 230 postmortem examinations from 13 farms indicate: 8% of kids died between 0-48 hours of age, 80%, between 48 hours and 40 days, and 12% between 40-120 days. Cause of death varied by age group. Overall causes were pneumonia-40%,

septicemia-21%, starvation-16%, diarrhea-8%, prematurity-6%, trauma-2%, congenital defects-1%, unknown-1% and 0.7% each for abomasitis, disbudding injury/encephalitis/meningitis, abomasal impaction, and polyserositis. Bacteria isolated from pneumonia and septicemia cases included *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Mycoplasmaspp.*, and *Coxiella burnetii*.

**Conclusions:** Preliminary results indicate pneumonia and septicemia are the most prevalent causes of death for kids up to 4 months of age in Ontario herds. The majority of deaths were kids over 48 hours old.

### **N-30: NECROTIZING MENINGOENCEPHALITIS ASSOCIATED WITH PARVOVIRAL INFECTION IN CATS**

Daniel Rissi, Grazieli Maboni, Anna Kokosinska, Jeremiah Saliki, Susan Sanchez

**Background:** Cerebellar hypoplasia is the most common neurologic disorder associated with perinatal or neonatal feline panleukopenia virus (FPV) infection in cats.

**Objective:** To characterize two cases of necrotizing meningoencephalitis associated with FPV in cats.

**Results:** An 8-week-old female domestic shorthaired cat (case 1) and a 5-month-old male domestic shorthaired cat (case 2) were autopsied after exhibiting acute ataxia (cases 1 and 2) and nystagmus (case 1). Both cats were part of a group of three cats sharing a room in a veterinary clinic. Cerebellar herniation through the foramen magnum, with flattening of cortical gyri and narrowing of sulci were present in both cats. Histologically there was lymphoplasmacytic meningoencephalitis with microglial nodules, neuronal necrosis in the cerebral cortex, hippocampus, and vestibular and abducens nucleus, and dilation of the periaxonal spaces with axonal spheroids and digestion chambers along the brainstem. Fluorescent antibody testing (FAT) for parvovirus on brain tissue was positive in both cases. Viral antigen was detected within neurons and endothelial cells via immunohistochemistry (IHC) in both cases. PCR using frozen brain tissue (case 1) was positive for parvovirus NS1 and VP2 genes; DNA sequencing and phylogenetic analysis revealed that the virus in these cats appears to be closely related with FPV detected in cats from Italy and Japan.

**Conclusions:** Neuropathological changes were associated with parvoviral infection in these two cats. DNA sequencing and phylogenetic analysis revealed the viral strain was more closely associated with FPV. Although uncommon, parvoviral infection should be considered in cases of necrotizing meningoencephalitis in cats.

### **N-31: ORAL FIBRO-PAPILLOMA IN LAMBS AND KIDS AFFECTED BY CONTAGIOUS ECTHYMA (ORF)**

Maria Giovanna Cancedda, Davide Pintus, Giantonella Puggioni, Gessica Tore, Rosario Scivoli, Angela Maria Rocchigiani, Elisabetta Coradduzza, Simona Macciocu, Antonio Lavazza, Ciriaco Ligios

**Background:** Contagious ecthyma (ORF) is a disease affecting sheep and goats caused by a virus belonging to the genus Parapoxvirus. In animals, ORF virus infection

is clinically characterized by the presence of pustules and vesicles as well as, sporadically, by productive lesions which are described as papilloma-like. Interestingly, ORF virus infection has been also described in man associated with rare tumor conditions of the skin.

**Objective:** To characterized by virological and pathological examination oral tumor-like lesions in lambs and kids affected by ORF virus.

**Methods:** In different Lacaune sheep flocks and Sarda goat herds, new-born lambs and kids showing clinical signs of ORF virus infection were investigated by virological and pathological examination.

**Results:** Grossly, we observed hyperemic verrucous growths appearing particularly along the gingival and lingual mucosa. Lesions were microscopically characterized by hyperkeratosis and hyperplastic epithelium with extremely elongated rete ridges, ballooning degeneration, and eosinophilic inclusion bodies. Proliferation of mesenchymal cells were also observed in the dermis. PCR technique determined Parapoxvirus DNA and excluded Papillomavirus (PV) presence in the mucosa tissue samples collected from all the examined animals. In addition, electron microscopy exclusively showed viral particles consistent with Parapoxvirus.

**Conclusions:** Our study reveals that in lambs and kids, ORF virus may be associated with oral fibro-papilloma. Although, PV is considered to be the most important etiological agent of fibro-papilloma, other viruses should be investigated in order to precisely determine the etiological agents implicated in the pathogenesis of oral papillomatous growths in animals.

## **N-32: IS FELINE TRICHURIASIS ASSOCIATED WITH LARGE INTESTINAL NODULES?**

Judit Magnusson Wulcan, Michelle Dennis, Jennifer Ketzis

**Background:** Trichuriasis can cause mucohemorrhagic diarrhea and weight loss in other species but there are no published studies about the condition in cats. More information on the consequences of infection is needed to guide clinical decision making, as the prevalence appears to be on the rise in areas previously relatively unaffected. Large intestinal nodules have been observed in *Trichuris* positive St. Kitts cats during necropsies performed for nematode collection. The nature of the nodules and whether they are associated to trichuriasis have not been investigated.

**Objective:** To investigate whether *Trichuris* infection is associated with large intestinal nodules in St. Kitts cats.

**Methods:** Necropsies were performed in 32 consecutive feline mortalities. Samples were collected for total worm counts and histopathology. The sample size was predetermined to be able to detect a strong association.

**Results:** *Trichuris* worms were grossly observed in approximately 40% of the cats (total worm counts are underway). Approximately 50% of the cats had multifocal small,

slightly raised, well demarcated, round, off-white submucosal nodules in the large intestine (histopathology is underway). The lesion frequency was higher in *Trichuris* positive than *Trichuris* negative cats, but the difference was not statistically significant. Most of the *Trichuris* positive cats were in good body condition, had formed feces and no grossly appreciable colitis.

**Conclusion:** Feline trichuriasis is not strongly associated with large intestinal nodular lesions, nor does there seem to be an apparent association with colitis, diarrhea or poor body condition.

### **N-33: REFERENCE CENTER OF PATHOLOGY FOR ARABIAN HORSES IN YAZD-IRAN**

Abelardo Morales

**Background:** The equine industry in the Middle East has recently expanded, increasing the number of competitions and improving the quality of horses, including Arab and Thoroughbred breeds. Current cases of mortality in the Arab breed are followed in a particular way in some establishments in the Middle East, but general data is not collected. These cases have made it possible to integrate clinical examination, pathology, and toxicological findings in racetrack horses in the Middle East.

**Objective:** The aim of this study was to describe the Reference Center of Pathology in Arabian Horses in Yazd-Iran, an innovative project with the purpose of studying the causes and associated factors of mortality in Arabian horses in the Middle East.

**Methods:** This project includes: 1) creation of a database of causes of mortality in Arabian horses from all participating countries; 2) creation of support service for the diagnosis of diseases in Arabian horses; 3) establishment of a reference center for the diagnosis; and 4) education and training of veterinary students in all participating countries.

**Results:** This project will generate great information about the diseases of horses at local and regional levels. Additionally, we can count on an updated information system of diseases of horses (diseases report update) and foreign substances identified post-race.

**Conclusion:** We developed the Reference Center of Pathology in Arabian Horses in Yazd-Iran, an innovative project with the purpose of studying the causes and associated factors of mortality in Arabian horses in the Middle East.

### **N-35: INVESTIGATION OF EPHA2 BIOMARKER EXPRESSION IN CANINE BRAIN CANCER**

Adam Whitelock, Emily Jones, Hannah Diebold, Hoi Ching Leung, Simon Puttick, Rachel Allavena

**Background:** Intracranial neoplasms remain some of the most devastating and difficult to treat cancers of both human and veterinary patients. Canine models are increasingly being recognized as superior analogues for human neoplasms, due to the spontaneous

development of neoplasms and greater protein expression homology. The biomarker EphA2 is involved in central nervous system development, angiogenesis and axonal repair, and has recently been found to be upregulated during neoplastic events.

**Objectives:** To Investigate the expression of EphA2 in canine intracranial neoplasms and characterise biomarker expression in normal canine tissues.

**Methods:** A retrospective analysis of 60 canine brain tumor samples (16 astrocytomas, 15 oligodendrogliomas, 6 glioblastomas, 23 meningiomas) and several normal canine tissues was conducted. Immunohistochemistry was conducted on brain tumor samples collected by the University of Queensland School of Veterinary Science during routine necropsy using a rabbit polyclonal EphA2 antibody ((C-20) SC-924,1:200 dilution). Canine stomach and endothelium was selected as a control as these have known positive expression in humans. Light microscopy was used to grade staining on intensity (0-3) and percentage stained (0%-100%).

**Results:** EphA2 was characterized to be highly expressed and ubiquitous throughout normal canine tissues. Normal brain tissue showed marked neuronal, ependymal and meningeal EphA2 expression, with minimal glial staining. Canine astrocytoma and glioblastoma multiforme samples demonstrated decreased EphA2 immunoreactivity, while oligodendrogliomas showed variable staining. Meningiomas demonstrated the most uniform, moderate expression of EphA2.

**Conclusions:** The biomarker EphA2 was found to be highly expressed and promiscuous in normal canine tissues, while being downregulated during intracranial neoplastic events.

### **N-36: PREVALENCE OF MYCOPLASMAS IN THE LOWER RESPIRATORY TRACT OF RACING THOROUGHBRED HORSES IN THE EAST ANGLIA REGION OF THE UNITED KINGDOM**

Alejandro Suarez-Bonnet, Emma Dixon, Alastair Foote, Andrew Rycroft, Simon Priestnall

**Background:** Inflammatory airway disease (IAD) is an important disorder that significantly limits racehorse performance and is usually detected by tracheal wash (TW) sampling. IAD is associated with both infectious and non-infectious causes. Various bacteria have been associated with inflammation in TW samples, with *Mycoplasma equirhinis* and *M. felis* reported infrequently, however the true prevalence of *Mycoplasma* spp. has not been thoroughly investigated.

**Objective:** To investigate the prevalence and identity of *Mycoplasma* species in TW samples of racehorses by PCR and sequence analysis.

**Methods:** Fresh TWs from 150 Thoroughbreds were obtained; 1) an aliquot of fresh TW, 2) a 1:1 dilution of TW cultured up to 10 days in modified-Friis liquid culture-medium, and 3) as 2) but with the TW sample filtered before culture. Extracted DNA was used for PCR with two primer sets against the 16S rRNA gene. Positive samples

were visualized by gel electrophoresis, PCR products were sequenced and results compared with published data by BLASTn search.

**Results:** 5.2% (3/58) fresh TW, 67.6% (46/68) cultured TW and 15.9% (13/82) cultured and filtered TW samples were PCR-positive for *Mycoplasma* spp. In 19 positive samples, sequencing identified five *Mycoplasma* species, the most frequent: *M. equirhina* (57.9%), *M. pulmonis* (21.1%) and *M. felis* (10.5%).

**Conclusions:** Mycoplasmas are frequently detected in TW samples from horses and culture is required to improve the sensitivity of PCR testing. *M. equirhina* and *M. felis* are present, but we also highlight the potential role of the rodent, and reportedly zoonotic, pathogen *M. pulmonis* in IAD.

### **N-37: NATURALLY OCCURRING AGE-RELATED, ALZHEIMER'S-LIKE PATHOLOGY IN AFRICAN GREEN MONKEY BRAINS**

Tatiana Corey, Oscar Illanes, Matthew Lawrence, Shervin Liddie, John Callanan

**Background:** The identification of natural animal models of Alzheimer's disease (AD) is critical to the development and preclinical evaluation of treatments. Prior studies have supported the St. Kitts African green monkey (AGM) as a relevant AD model. With its abundant population, low zoonotic disease burden, and evidence that aged AGMs develop amyloid plaques, this model warrants further evaluations, including comprehensive characterization of background pathology and assessment of age-related neuropathology.

**Objective:** Define the background and age-related neuropathology of AGMs.

**Methods:** Brains of 71 AGMs were examined histologically, representing age cohorts of 3-6 years (n=20), 7-9 years (n=20), 10-15 years (n=20), >16 years (n=11). A subset of brains from each cohort (n=31) were assessed immunohistochemically for AD-related pathology using 4G8 Beta-amyloid and GA5 GFAP antibodies.

**Results:** Frequently observed age-related microscopic findings included white matter and neuropil vacuolation and neuronal lipofuscinosis. Less common findings included perivascular calcification, spheroid formation, and focal gliosis. Immunohistochemistry revealed 4G8-positive amyloid plaques in the prefrontal, frontal, and temporal cortices of 9 of 11 animals over 16 years old; additional plaques were observed in the entorhinal cortex of one of these geriatric monkeys. Many plaques were co-localized with increased GFAP immunostaining.

**Conclusions:** Background neuropathology findings, often subtle, were present in monkeys of all ages. Some findings were more evident with age and should be considered when interpreting neuropathology in future studies. AD-related pathology was clearly age-related and common in geriatric AGMs over 16 years old. These findings highlight the value of the AGM as a natural model of AD.

### **N-38: VALIDATION AND QUANTIFICATION OF BETA-2 ADRENORECEPTOR IMMUNOHISTOCHEMISTRY IN CANINE SPLENIC HEMANGIOSARCOMA**

Samantha Schlemmer, Jesse Dawson, Heather Wilson-Robles, Raquel Rech, Mark Johnson, Andy Ambrus, Kristin Armstrong

**Background:** Hemangiosarcoma (HSA) is an aggressive neoplasm of endothelial cells that commonly affects the canine spleen. Beta-adrenoreceptor expression has been documented in human vascular tumors and canine HSA cell lines. Beta-adrenoreceptor antagonists have been reported to suppress endothelial cell proliferation, migration, and differentiation, and may serve as anticancer drugs for vascular tumors.

**Objectives:** (1) Assess  $\beta$ 2-adrenoreceptor ( $\beta$ 2AR) expression via immunohistochemistry (IHC) in normal formalin-fixed paraffin-embedded (FFPE) canine cardiac and splenic tissue; (2) assess  $\beta$ 2AR expression in FFPE canine splenic HSA specimens; (3) correlate  $\beta$ 2AR IHC findings with patient data to determine if it is predictive of patient outcome and/or can serve as marker for adrenergic antagonist therapy.

**Methods:**  $\beta$ 2AR IHC protocol was optimized on normal canine cardiac and splenic tissue using CD31 as a control marker for endothelial cells. Sixty-nine surgically obtained canine splenic HSA specimens were subjected to  $\beta$ 2AR IHC. Ten random 40x fields of tumor are being assessed for immunoreactivity using ImageJ software. IHC expression will be evaluated using a one-way ANOVA with Bonferroni post hoc analysis. Case characteristics will be evaluated using standard means along with a correlation analysis. Kaplan-Meier curves will be used to assess survival differences between groups. A p value <0.05 will be considered statistically significant.

**Results/Conclusions:**  $\beta$ 2AR is expressed in the tunica media of normal canine cardiac and splenic arterioles, displaying positive cytoplasmic immunoreactivity.  $\beta$ 2AR is expressed in the cytoplasm of neoplastic endothelial cells in canine splenic HSA. Image analysis and statistics are pending before the hypotheses can be accepted or rejected.

### **N-39: DETECTION OF MYCOPLASMA SP. IN RHESUS MONKEYS (MACACA MULATTA) FROM BRAZILIAN LABORATORY COLONY – PRELIMINARY DATA**

Anna Mongruel, Cássia Duquia, André Somma, Fabiano Montiani-Ferreira, Ana Pinto, Carla Campos, Mônica Calado, Maria Lara, Jessica Valente, Thállitha Vieira, Rafael Vieira

**Background:** Rhesus monkeys (*Macaca mulatta*) are found in temperate forests of India, China, and Afghanistan and have demonstrated a high level of adaptability. In Brazil, this non-human primate species is restricted to zoos and controlled areas and is commonly used as an experimental model for human diseases. Previous studies have demonstrated that hemoplasmas should be considered as a confounding factor in experiments performed on laboratory animals. Three hemoplasma species are known to infect non-human primates: *Candidatus Mycoplasma kahanei*, *Ca. Mycoplasma haemomacaque* and *Ca. Mycoplasma aoti*. Additionally, a potentially novel *Mycoplasma* sp. was detected in *Sapajus* sp. from Brazil.

**Objective:** To screen a Brazilian laboratory colony of rhesus monkeys for hemoplasma infection by polymerase chain reaction (PCR) based assays.

**Methods:** Blood samples from eight rhesus monkeys were taken, DNA extracted, and samples were screened by a pan-hemoplasma PCR targeting a fragment of the 16S rDNA gene of *Mycoplasma* sp.

**Results:** All samples consistently amplified the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Five out of eight (62.5%; 95% CI: 30.5-86.3%) rhesus monkeys were positive for *Mycoplasma* sp. by PCR. Preliminary sequencing data suggests that animals were infected by *Ca. M. haemomacae*.

**Conclusions:** This is the first study evaluating hemoplasma infection in a laboratory colony of non-human primates. Further studies are needed to evaluate the impact of *Mycoplasma* sp. infection on the experiments involving these non-human primates.

#### **N-40: IMMUNOHISTOCHEMICAL EXPRESSION OF CXCR4 IN CANINE BRAIN CANCER**

Hoi Ching Leung, Emily Jones, Adam Whitelock, Hannah Diebold, Simon Puttick, Rachel Allavena

**Background:** Brain cancer has one of the worst prognoses of all cancers in both humans and dogs. Novel diagnostic and therapeutic tools are needed for the advancement of brain cancer prognosis and treatment.

**Objectives:** To investigate the expression of CXC chemokine receptor type 4 (CXCR4) in canine brain tumors as a novel biomarker and a potential target for the development of theranostics for human and canine brain cancers.

**Methods:** Sixty canine brain tumor samples, including 23 meningiomas, 6 glioblastomas, 16 astrocytomas and 15 oligodendrogliomas were selected from the archive of the diagnostic pathology service at the University of Queensland School of Veterinary Science. The expression of CXCR4 was examined using immunohistochemistry (IHC) with anti-CXCR4 anti-rabbit polyclonal antibody. Canine skin was used as a positive control. Various normal canine tissues, including brain, were also stained using IHC for characterization of tissue specific expression of CXCR4.

**Results:** Normal canine brains exhibited strong endothelial and meningeal staining, moderate neuropil staining, and mild to moderate cytoplasmic staining in astrocytes, oligodendrocytes and neurons. In tumor samples, astrocytoma and glioblastoma cells exhibited mild cytoplasmic staining, while meningioma cells exhibited strong cytoplasmic staining. Negative or variable staining was observed in oligodendrogliomas. Strong endothelial staining was observed in all tumor types.

**Conclusion:** Different levels of expression in different canine brain tumor types were observed. Further investigation will differentiate expression in each tumor type to provide further information on the use of CXCR4 in dogs as a brain tumor biomarker.

## **N-41: SALSA AND EQUINE ASTHMA**

Gary Lee, Laurence Tessier, Dorothee Bienzle

**Background:** Salivary scavenger and agglutinin (SALSA, also known as deleted in malignant brain tumors 1 or DMBT1) is a protein with putative functions in innate immunity and tissue repair. In humans, the protein has been localized mainly to mucosal epithelia and secretions, including those of the airways. Knowledge regarding SALSA in horses is limited, but transcriptomic analysis of bronchial biopsies indicated low expression during remission and exacerbation in asthmatic relative to non-asthmatic horses.

**Objectives:** Our objectives were to determine the sequence of the SALSA gene and to characterize tissue expression in horses.

**Methods:** SALSA gene from bronchial cDNA samples of multiple horses was amplified and sequenced. Tissue microarrays from 3 horses containing 21 tissues each were constructed. Immunohistochemical assays for SALSA were validated and applied to equine tissue microarrays.

**Results:** The gene in horses includes multiple scavenger receptor cysteine-rich (SRCR) domains, SCRC-interspersed domains (SID) and one CUB (C1r/C1s, uegf, bmp-1) domain. These domains mediate microbial agglutination and the binding of ligands such as those involved in innate immunity. The number of SRCR domains varied between horses, but overall sequence identity between horse and human was approximately 75%. SALSA was highly expressed at mucosal sites, including bronchial and bronchiolar epithelium, mucosal cells of the duodenum and the transitional cells of the urinary bladder.

**Conclusions:** SALSA is a multifunctional protein with multiple isoforms in different individuals with a predilection for mucosal cells. Future studies will delineate the role of SALSA in enhancing or attenuating neutrophilic inflammation in the airway epithelium of horses.

## **N-42: BRUCELLA CETI-CELLULAR PRION PROTEIN INTERACTION IN THE BRAIN FROM NEUROBRUCELLOSIS-AFFECTED STRIPED DOLPHINS (STENELLA COERULEOALBA): AN IN-SILICO STUDY**

Giovanni Di Guardo, Gabriella Di Francesco, Ludovica Di Renzo, Manuela Tittarelli, Cristina Esmeralda Di Francesco, Marina Baffoni, Carla Grattarola, Sandro Mazzariol, Cinzia Centelleghes, Leonardo Leonardi, Antonio Petrella, Massimiliano Orsini

**Background:** *Brucella ceti* is recognized as a cause of central nervous system (CNS) inflammation in striped dolphins (*Stenella coeruleoalba*), with stranding and death of neurobrucellosis-affected animals. The marked neurotropism of *B. ceti* in *S. coeruleoalba* made us hypothesize a pathogenetic role of host's cellular prion protein (PrP<sup>C</sup>), the expression levels of which are significantly higher in the CNS. Previous work showed that *B. abortus* entry into murine macrophages is mediated by bacterial HSP60-cellular membrane PrP<sup>C</sup> interaction.

**Objectives:** In order to assess the biological plausibility of the interaction between *B. ceti* HSP60 and striped dolphin's PrP<sup>C</sup>, we performed an *ad hoc in-silico* study.

**Methods:** Sixteen (top 2%) *B. ceti* HSP60-*S. coeruleoalba* CNS cell PrP<sup>C</sup> "interaction solutions" were revealed by this bioinformatics approach, with 4 of them appearing to be more plausible than the others.

**Results and conclusion:** The herein presented data, albeit original, needs laboratory confirmation. In this respect, while work is currently underway, it should be additionally emphasized that high tissue levels of dioxin-like compounds were measured in at least 1 out of 5 *B. ceti*-infected, neurobrucellosis-affected striped dolphins found stranded in recent years along the Ionian coast of Apulia Region (Southern Italy). This is of interest, given the pro-apoptotic activity exerted by dioxins, versus the anti-apoptotic role of PrP<sup>C</sup>, which could lead to a dioxin-driven, increased expression of PrP<sup>C</sup> in the CNS from these dolphins and, consequently, to a more rapid progression of their CNS colonization on behalf of *B. ceti*.

#### **N-43: ACUTE ACCIDENTAL EXPOSURE TO UNDILUTED QUATERNARY AMMONIUM IN DOGS**

Sonika Patial, Tatiane Watanabe, Raphael Malbrue, Carmen Arsuaga, Rhett Stout, Ingeborg Langohr, Nobuko Wakamatsu

**Background:** Two adult female hound dogs with a history of severe respiratory distress and depression were submitted for postmortem examination after accidental exposure to Quaternary Ammonium Compounds (QACs). QACs are amphoteric cationic detergents widely used as adjuncts to industrial, medical, and domestic environments and are commonly found in disinfectants. The undiluted disinfectant was reportedly attached to a proportioner by tubing that came loose and fell to the lower side creating a siphoning effect. The undiluted disinfectant ran through dog runs and two exposed dogs were found early in the next morning.

**Objective:** To determine gross and histological findings relevant to QAC exposure.

**Methods:** Postmortem examination was performed.

**Results:** Major gross findings in both cases consisted of severally swollen faces and necks, petechiae on the inguinal region, and partial sloughing and multifocal erosions and ulcerations of the lingual mucosa. Histologically, there was severe coagulative necrosis of the exposed skin and multiple mucosal surfaces.

**Conclusions:** The QAC disinfectant is normally proportioned at 1:256 and is applied to spaces with no animals present. MSDS indicates the chemical as a corrosive and it is listed as a health hazard by the National Fire Prevention Association with a rating of 3. To our knowledge, these ratings are applied only to the undiluted compound. This case was reported to the Office of Laboratory Animal Welfare (OLAW) as required by the universities assurance statement on file at OLAW. The report contained a complete description of the event, causes, outcomes, and a plan for prevention moving forward.

#### **N-44: ANAPLASTIC LARGE T-CELL LYMPHOMA IN THE INTESTINE OF DOGS**

Lauren Stranahan, Derick Whitley, Tuddow Thaiwong, Matti Kiupel, Fabiano Oliveira

**Background:** Anaplastic large T-cell lymphoma (ALTCL) is a rare subtype of non-Hodgkin's T-cell lymphoma that occasionally occurs in the gastrointestinal tract of humans. Enteropathy-associated T-cell lymphoma (EATL) type I is the most common type of intestinal lymphoma in dogs and ALTCL has not previously been reported in the intestinal tract of dogs.

**Methods:** Thirteen dogs with intestinal masses diagnosed as intestinal lymphoma with anaplastic morphology were reviewed. Clinical data, including treatment protocols, were available for 11 dogs. Immunohistochemistry for CD3 and CD20 was performed in all cases in addition to PCR for Antigen Receptor Rearrangements (PARR) for assessment of clonality.

**Results:** Eight (61.5%) of the dogs presented with intestinal perforation and all cases had one or more masses arising from the small intestine. Histologically, all cases were characterized by transmural infiltrates of large-sized, CD3-positive T cells with prominent nucleoli. Neoplastic T cells exhibited marked anisocytosis and anisokaryosis and had occasionally indented to reniform nuclei. Ischemic necrosis due to vascular damage caused by vasoinvasion of neoplastic cells with secondary inflammation were common findings. All cases had a monoclonal T-cell receptor gamma gene rearrangement. The median survival time was 5 days with one dog surviving 2 years after the initial diagnosis.

**Conclusions:** ALTCL can occur as an aggressive transmural lymphoma in the gastrointestinal tract of dogs and commonly causes intestinal perforation, possibly due to ischemia of the affected segment. ALTCL must be differentiated from EATL type 1 for accurate prognostication and selection of therapeutic options.

#### **N-45: MICRORNA EXTRACTION FROM FORMALIN-FIXED PARAFFIN-EMBEDDED CANINE LYMPH NODES: QUANTITY AND QUALITY ASSESSMENT USING THREE COMMERCIAL EXTRACTION KITS**

Mara Varvil, Nelly Elshafie, Andrea Dos Santos

**Background:** MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and are altered in cancer. Expression profiles of miRNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues correlate with fresh-frozen specimens; however, there is a paucity of studies reporting on the quantity and quality of miRNAs isolated from FFPE lymph nodes in dogs.

**Objectives:** Compare commercial kits for miRNA extraction from FFPE lymph nodes and evaluate viability.

**Methods:** Total RNA was extracted from FFPE lymph nodes from non-neoplastic tissues using QIAGEN miRNeasy FFPE kit (MRN), Invitrogen Purelink FFPE kit (INV), and Agilent Absolutely RNA FFPE Kit (ABS). MiRNAs from FFPE lymphomas were extracted with MRN. Quantity and quality of total RNA was assessed by UV

spectrophotometry (NanoDrop), fluorometry (Qubit 4), and electrophoretic assay (Agilent 2100 Bioanalyzer, Agilent RNA 6000 Nano Kit). RT-qPCR (miScript RT-qPCR system, QIAGEN) was performed in non-neoplastic and lymphoma samples.

**Results:** For MRN, INV and ABS, respectively, the total time per extraction batch was 4, 4:30, and 14:45 h, the average total RNA concentration was 229.9, 283.0, and 156.3 ng/uL, and the average 260/280 ratios were 1.95, 1.96, and 1.99. Fluorometry evaluation was comparable to Nanodrop, while the Agilent Bioanalyzer was not useful to assess quality. The samples verified by RT-qPCR expressed the microRNAs tested.

**Conclusions:** Quantification by Nanodrop is useful in determining total RNA concentration. RT-qPCR is needed to demonstrate the presence of amplifiable miRNA. MiRNAs can be extracted from FFPE lymph node tissues, allowing miRNA expression studies in archived canine lymph node samples.

#### **N-46: THE ROLE OF NUCLEAR FACTOR-KAPPA B IN FELINE INJECTION SITE SARCOMAGENESIS**

Cheng-Shun Hsueh, Ching-Ho Wu, Cheng-Hsin Shih, Lih-Seng Yeh, Chian-Ren Jeng, Fei Pang, Hue-Ying Chiou, Hui-Wen Chang

**Background:** Chronic inflammation has been implicated in sarcomagenesis. Among various factors, activation of nuclear factor-kappa B (NF- $\kappa$ B) signaling pathway has been documented being able to target genes associated with tumor progression and up-regulate the expression of tumor-promoting cytokines and survival genes in several human tumors. Feline injection-site sarcomas (FISS) are malignant neoplasms of mesenchymal origin that have been proven to be associated with vaccine adjuvant, aluminum, which serves as a stimulus continuously inducing overzealous inflammatory and immunologic reactions.

**Objective:** To understand the contribution of NF- $\kappa$ B in the oncogenesis of FISS.

**Methods:** In this study, detection of activated form of NF- $\kappa$ B in paraffin-embedded specimens in 39 cases, *in vitro* establishment of primary cells derived from FISS, and the functional effects of the NF- $\kappa$ B inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), on primary tumor cells were conducted.

**Results:** We showed that activated form of NF- $\kappa$ B was detected in 88.5% of FISS cases, suggesting that NF- $\kappa$ B might play a role in the sarcomagenesis of FISS. Three primary cells derived from FISS of three cats exhibiting similar immunohistochemical characteristics have also been established. FISS primary cells treated with DHMEQ showed growth inhibitory effects with a 50% cell growth inhibition (IC<sub>50</sub>) concentration of approximately 17  $\mu$ g/ml and apoptosis in dose-dependent manner.

**Conclusions:** The primary tumor cells could be used for preclinical evaluation of therapeutic drugs for FISS. Moreover, dose-dependent inhibitory effects on the growth of FISS primary cells treated with DHMEQ suggested that NF- $\kappa$ B might be a new molecular therapeutic target for FISS.

## **N-47: EXAMINING THE GENETIC BACKGROUND OF CANINE PHEOCHROMOCYTOMAS**

Firas Abed, Michael Dark

**Background:** Pheochromocytomas (PCs) are tumors originating from the adrenal medullary chromaffin cells. Based on genetic mutations, human PCs are classified into two main clusters. Cluster I represents the pseudo-hypoxic pathway, while cluster II represents mutations with abnormal kinase signaling pathway activation. In humans, gene sequencing and immunohistochemistry (IHC) effectively predicts behavior and likelihood of recurrence. However, these tools have not been used in canine medicine.

**Objective:** The current study aims to determine the role of genetics in canine pheochromocytoma pathogenesis and its effects on prognosis and tumor behavior.

**Methods:** Using the Nextera Human Exome Enrichment Kit for canine exon sequencing and performing SDHA and SDHB immunohistochemistry on 24 canine formalin-fixed paraffin embedded (FFPE) PCs.

**Results:** A total of 88.35% of reads processed mapped to canine exons and 11.65% mapped to introns, with an average sequencing depth of 3X. Out of 18 samples with positive SDHA immunoreactivity, 12 lacked SDHB immunoreactivity, suggesting SDH-family mutations excluding SDHA. Interestingly, we had lost of immunoreactivity for both SDHA and SDHB in four samples, suggesting mutation in SDHA. These data suggest that 16 out of the 24 (66.7%) samples may have an SDH family mutation.

**Conclusions:** Human exome sequencing kits may be useful in determining mutations in canine neoplasms. SDH immunohistochemistry appears useful in focusing efforts to determine the genetic basis of canine PCs. Further work will combine these results to determine the genetic basis of PCs in dogs.

## **N-48: IMMUNOHISTOCHEMICAL EVALUATION OF P53 EXPRESSION AND SEQUENCING OF GENOMIC DNA IN MALIGNANT CANINE INTESTINAL TUMORS**

Seung Hee Cho, Byung Joon Seung, Soo Hyeon Kim, Jung Hyang Sur

**Background:** p53 has an important role in the induction of programmed cell death (apoptosis). The rapid cycling of cells in the intestine is important, and p53 plays an important role in this process. p53 protein is mutated in many tumors, in which cases overexpression of p53 is observed. It is known to be mutated at the stage of development in intestine tumor, especially from benign to malignant.

**Objective:** To determine prevalence of p53 tumor suppressor protein overexpression in intestinal tumors of dogs, and gene mutation in cases with overexpression of p53.

**Methods:** Immunohistochemical analysis for p53 was carried out in formalin-fixed, paraffin-embedded sections of tissues in intestine from 30 dogs who underwent surgery or biopsy, by the standard peroxidase-antiperoxidase method. Expression of this protein in benign (polyps and adenomas, 14 cases) and malignant (adenocarcinomas, 16

cases) tumors was compared. DNA was extracted from FFPE in 10 cases with overexpression of p53, and sequencing for exon 4 to exon 8 was performed.

**Results:** Increased p53 expression was observed in malignant tumors when compared to benign lesions ( $p=0.024$ ). Sequencing of 10 p53 overexpressed cases showed no mutation in exon 4 to 8.

**Conclusion:** p53 was found to be overexpressed in malignant tumors, but no mutation was found in overexpressed cases. This study suggests that the expression of p53 may be influenced by other reasons than the gene mutation, or that there is changes in the upstream regulators of p53.

#### **N-49: SALIVARY GLAND PATHOLOGY IN DOGS: A RETROSPECTIVE STUDY OF 174 CASES**

Danielle Lieske, Daniel Rissi

**Background:** Salivary glands are an uncommon site of biopsy sampling in veterinary practice, which is reflected by the scarcity of published data describing the pathologic changes affecting these glands.

**Objective:** The aim of this study was to characterize and identify common diseases and conditions associated with salivary gland biopsies.

**Methods:** In this retrospective study, we evaluated tissue changes in 174 salivary gland samples from dogs submitted to our surgical biopsy service between 2010 and 2017.

**Results:** The most common reasons for submission were regional swelling and/or suspected sialoceles and halitosis. Out of the 174 cases, no pathological changes were detected in 42 samples (24.1%); nonspecific inflammation was noted in 89 cases (51.1%), and consisted of lymphoplasmacytic, neutrophilic, or granulomatous infiltrates with no evident cause; necrotizing sialometaplasia was diagnosed in 3 cases (1.7%); lipomatosis and hemorrhage were diagnosed in 4 cases (2.3%) and 1 case (0.57%), respectively. Neoplasia was diagnosed in 35 cases (20%); of these, 27 (77.1%) had epithelial origin, 1 (2.9%) had mesenchymal origin, and 1 (2.9%) had round cell origin. The tumor histogenesis could not be determined in 6 cases (17.1%).

**Conclusions:** This study highlights the high frequency of inflammatory changes possibly associated with sialoceles and the large number of malignant epithelial neoplasms over other types of neoplasia. Salivary gland submissions comprised 0.3% of 59,698 canine biopsy submissions to our laboratory within the studied period. Most cases were submitted because of local swelling near the salivary glands, and a surprising number of tissues had no discernible pathological changes.

#### **N-50: HISTOLOGICAL FEATURES OF AUSTRALIAN CANINE AND FELINE BLADDER DISEASE**

Emily Jones, Chiara Palmieri, Mary Thompson, John Al-Alawneh, Karen Jackson, Rachel Allavena

**Background:** Bladder diseases cause high clinical morbidity and occasional mortality in cats and dogs, but their pathological features are understudied.

**Aims:** To investigate the histopathological changes of bladder diseases in cats and dogs, and develop firm case definitions and classifications for these conditions.

**Methods:** Canine and feline cases with bladder tissue archived at The University of Queensland School of Veterinary Science (UQSVS) and the Murdoch University School of Veterinary and Life Sciences (MUSVLS) were included in this retrospective study. Logistic regression was used to measure association between histopathological changes using light microscopy, and diagnostic outcomes.

**Results:** There were 339 canine and 98 feline bladder cases recorded in the UQSVS database from 1994-2016 with the following diagnostic outcomes: 124 cystitis, 75 neoplasia, 14 urolithiasis, 51 miscellaneous (peritonitis/ haemorrhage/ autolysis), and 173 normal. Preliminary analysis of histological variables in bladder sections from UQSVS and MUSVLS showed that some variables were associated with the diagnostic process, while others were associated with the disease outcome. For disease outcome, cystitis (OR 4.98,  $P = 0.02$ ) and neoplasia (OR 4.55,  $P = 0.01$ ) cases were at least four times more likely to have increased numbers of submucosal inflammatory cells, and less likely to have serosal inflammation (OR 0.12,  $P = 0.01$ ) or serosal haemorrhage (OR 0.15,  $P = 0.014$ ) than the other outcomes.

**Conclusions:** Differences have been identified in some histopathological variables that can be associated with either the diagnostic procedure or the disease outcome. Ongoing analyses will interrogate these differences and determine their diagnostic value.

## **N-51: HISTOPATHOLOGICAL AND CLINICAL CHARACTERIZATION OF CANINE MENINGOENCEPHALITIS OF UNKNOWN ETIOLOGY**

Siobhan O'Sullivan, Robert Foster, Karen Vernau, Fiona James, Olaf Berke, Virginia Madsen, Stefan Keller

**Background:** Meningoencephalitis of unknown etiology (MUE) is a group of idiopathic inflammatory neurologic diseases of dogs, which includes the subtypes granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis (NME) and necrotizing leukoencephalitis (NLE). The pathogenesis of MUE is unknown but suspected to be multifactorial, and linked to genetics and autoimmunity. Small breeds are overrepresented, and despite immunosuppressive treatment the prognosis is guarded. Current clinical diagnosis is insufficiently specific to categorize MUE into subtypes of inflammatory disease, and postmortem histology of the brain to visualize the distribution of lesions provides the most reliable specific diagnosis.

**Objectives:** The long-term goal of this project is to develop a novel, minimally invasive, antemortem diagnostic tool to improve prognostication and treatment for clinical MUE. Our immediate objective is to characterize a series of MUE cases with respect to histological and clinical features for subsequent molecular analysis.

**Methods:** Histopathological specimens from patients with inflammatory brain diseases were reviewed, and 89 cases could be subtyped based on established histologic criteria for MUE (65 GME, 15 NME and 9 NLE) while 6 cases displayed atypical features. Clinical data was collated and reviewed for prognostic trends related to MUE subtype.

**Results:** GME was the most common MUE and exhibited the most variable survival times. Small breed dogs were overrepresented, reflecting breed predispositions, but medium to large breeds were also affected.

**Conclusions:** Forthcoming results of this ongoing study will include immune repertoire sequencing, analysis, and correlation of repertoires with histologic subtype and clinical parameters such as signalment, response to therapy or overall outcome.

## **N-52: BREED SPECIFIC DIFFERENCES IN MOLECULAR FEATURES AND CLINICAL OUTCOME IN CANINE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

Emily Rout, Julia Labadie, Robert Burnett, Janna Yoshimoto, Anne Avery

**Background:** Human B-cell chronic lymphocytic leukemia (B-CLL) demonstrates marked heterogeneity in clinical outcome and pathophysiology, but little is known about heterogeneity within canine B-CLL. An important prognostic factor in human B-CLL is immunoglobulin heavy chain variable (IGHV) gene mutation status. Human B-CLL patients with unmutated IGHV genes, having not undergone somatic hypermutation, have more aggressive disease than patients with IGHV genes that have undergone somatic hypermutation.

**Objectives:** Examine IGHV mutation status, clinical outcome and gene expression among canine B-CLL patients, comparing small-breed dogs (over-represented in B-CLL) and Boxer dogs.

**Methods:** We sequenced the IGHV genes of 55 canine B-CLL patients, including 36 non-Boxers and 19 Boxers, and used sequence homology with the reference genome to determine mutation status. Medical records from 42 small-breed and 18 Boxer B-CLL patients were reviewed. We used RNA-Seq to examine gene expression profiles among canine B-CLL cases, diffuse large B-cell lymphoma cases and normal B cells, including Boxer B-CLL (n=4) and small-breed B-CLL (n=8) cases.

**Results:** Boxer dogs preferentially used unmutated IGHV genes (79% of cases), while only 25% of non-Boxer cases were unmutated. Median overall survival was significantly shorter in Boxers (MST=5 months) than small-breed dogs (MST=19 months). Gene expression analysis segregated B-CLL cases from DLBCL cases, and showed heterogeneity amongst B-CLL samples. Differentially expressed genes in B-CLL versus control B cells were enriched for B-cell receptor and NF-KB signaling pathways.

**Conclusions:** Canine B-CLL has heterogeneous molecular features and clinical outcome. Boxers appear to preferentially use unmutated IGHV genes and have more aggressive disease.

### **N-53: DETERMINATION OF TREATMENT FAILURE FOR FIP INFECTED CATS TREATED WITH ANTICORONAVIRAL THERAPY TO FACILITATE THE SUCCESS OF SECOND GENERATION THERAPY**

Molly Liepnieks, Brian Murphy, Michel Perron, Michael Bannasch, Elizabeth Montgomery, Eisuke Murakami, Hongwei Liu, Niels Pedersen

**Background:** Utility of GS-441524 was evaluated in 31 naturally feline infectious peritonitis virus (FIPV) infected client-owned cats. 26 cats completed one or more rounds of treatment. To date (10-13 months post-study enrollment), 24 cats remain healthy. Seven cats were euthanized.

**Objective:** Determine the cause of treatment failure in naturally FIPV infected cats treated with anticoronaviral therapy in order to facilitate the success of second generation antiviral therapy.

**Methods:** Necropsies were performed on 6/7 cats that were euthanized 2-23 days following trial enrollment; one cat was lost to follow-up. Full necropsy with histopathologic examination was performed. FIPV immunohistochemistry was performed on selected tissues. Viral RNA transcripts were quantified by qRT-PCR from ascites fluid when available.

**Results:** Four cats were found to have FIPV lesions; three in the central nervous system and one restricted to the abdomen. No evidence of FIPV infection was identified in two cats; one with a gastric perforation and one with thromboembolic disease secondary to congenital hypertrophic cardiomyopathy (HCM). Of four cats euthanized due to FIPV infection, three had evidence of ongoing viral replication by IHC and/or qRT-PCR.

**Conclusions:** To date, 92% of cats completing treatment have been cured and data supports drug-associated inhibition of viral replication in vivo. Cats failing treatment often failed early, demonstrated ongoing viral replication, and neurologic involvement. Ongoing viral replication may represent viral resistance or host factors (inability to activate prodrug). Neurologic disease may require more aggressive or multimodal treatment. Additionally, early failures may represent more severe disease at time of treatment initiation.

## Clinical Pathology Focused Scientific Session I

November 4, 2018 | 1:30 PM –4:30 PM

Session Chair: Julie Webb, DVM, DACVP, IDEXX Laboratories, Markham, Ontario

November 4, 2018

1:30 PM – 1:45 PM

### **B1-TUBULIN GENE POINT MUTATION ASSOCIATED WITH CONGENITAL MACROTHROMBOCYTOPENIA IN CAIRN AND NORFOLK TERRIERS IDENTIFIED IN TWO JACK RUSSELL TERRIERS**

Pete Christopherson, Kevin King, Mary Boudreaux

Congenital macrothrombocytopenia is associated with mutations in over 20 genes in people and may or may not be associated with bleeding tendencies. In veterinary medicine, two distinct mutations associated with macrothrombocytopenia and no clinical bleeding have been identified in the gene encoding  $\beta 1$ -tubulin. Identifying patients with these mutations is important to rule-out the existence of immune-mediated or other forms of acquired thrombocytopenia. One is a point mutation at coding nucleotide 745 in exon 4 that is most commonly associated with Cavalier King Charles Spaniels. This mutation has also been documented in over 20 other dog breeds by our laboratory. The second is a point mutation at coding nucleotide 5 in exon 1 that is seen most often in Cairn and Norfolk Terriers. Recently, we identified two apparently unrelated Jack Russell Terriers (a 4-year-old male and a 3-year-old female) with persistent thrombocytopenia of ~50,000-80,000/ $\mu$ L with no evidence of abnormal hemorrhage. Examination of platelet morphology on fresh blood smears confirmed the existence of a macrothrombocytopenia. DNA isolated from EDTA blood from both dogs was subjected to PCR using primers designed to amplify the areas around the mutation sites in exons 1 and 4. Both dogs tested homozygous affected for the mutation in exon 1 and both tested clear for the mutation in exon 4. This finding, combined with the presence of the exon 4 mutation in expanding numbers of canine breeds, makes congenital macrothrombocytopenia a differential to consider for any dog with persistent macrothrombocytopenia in the absence of prolonged bleeding.

November 4, 2018

1:45 PM – 2:00 PM

### **CEREBROSPINAL FLUID ANALYSIS WITH THE PROCYTE DX HEMATOLOGY ANALYZER AND COMPARISON WITH MANUAL NUCLEATED CELL COUNTS AND DIFFERENTIALS**

Holly Brown, Jessica Wilson-Hess, Fred Metzger, Dennis DeNicola

**Background:** To avoid sample degradation of cerebrospinal fluid (CSF) samples collected in private practice, a simple, rapid, and accurate means of determining total nucleated cell counts (TNCC) and cell differentials is needed. In-clinic hematology analyzers could address these needs.

**Objective:** The aim of this study was to compare manual TNCC and nucleated cell differentials to that obtained by the in-clinic IDEXX ProCyt Dx Hematology Analyzer (PDx) on dog and cat CSF samples.

**Methods:** CSF samples were obtained from client-owned dogs (n=80) and cats (n=4) as part of their diagnostic work-up for neurologic disorders. Automated TNCC and nucleated cell differentials were determined by the PDx and compared to that determined by manual hemocytometer, sedimentation, and microscopy. Method comparisons were made with Spearman's correlation and Bland-Altman bias analyses.

**Results:** Manual hemocytometer-derived CSF TNCC ranged from 0-7736/ $\mu$ L. The majority (64/84, 76%) were <10/ $\mu$ L. The Spearman's correlation coefficient of 0.77 supports strong correlation between manual and PDx counting methods. Manual microscopic granulocyte and agranulocyte counts ranged from 0-6885/ $\mu$ L and 0-851/ $\mu$ L, respectively. The Spearman's correlation coefficient of 0.50 and 0.80, respectively supports strong correlation between manual and PDx leukocyte differentials. With TNCC below 300/ $\mu$ L, the mean bias was 2.9/ $\mu$ L, 1.4/ $\mu$ L and 1.2/ $\mu$ L for TNCC, granulocytes and agranulocytes, respectively.

**Conclusions:** Automated analysis of CSF on the PDx provides useful and reliable TNCC and nucleated cell differentials over a range of cell counts and cellular composition. As such, it is an effective tool that allows practices to derive important and timely diagnostic information from their in-clinic laboratory.

November 4, 2018

3:30 PM – 3:45 PM

### **EFFECT OF MICROSCOPIC AREA ON DIFFERENTIAL CELL COUNTS IN EVALUATION OF EQUINE BRONCHOALVEOLAR LAVAGE FLUID**

Kimberley Foote, Cornelia Gilroy, Shelley Burton, Raphael Vanderstichel

**Background:** Horses experience non-infectious respiratory disease divided into inflammatory airway disease (IAD) and recurrent airway obstruction (RAO) partly based on cytology of bronchoalveolar lavage (BAL) fluid samples. Differentiation of IAD and RAO is largely determined by neutrophil percentages on differential cell counts. 500-cell differential counts are typically performed on cytocentrifuged samples from randomly chosen areas of the microscopic field. Inconsistent results during repeat cell counts has raised concern for method reliability.

**Objective:** To determine if neutrophil percentages are affected by microscopic area examined.

**Methods:** 500-cell differential counts were performed on 54 equine BAL fluid samples using 3 methods - periphery, center and random areas of the cell dot. Neutrophil percentages were compared using concordance correlations ( $\rho_c$ ) and Bland-Altman analyses on  $\log_{10}$  transformed data. Statistical significance of mean differences between methods was based on exclusion of zero from the estimated confidence intervals.

**Results:** Concordance correlations were high between each pair of methods ( $\rho_c$  (random vs periphery) = 0.90;  $\rho_c$  (random vs center) = 0.93;  $\rho_c$  (periphery vs center) = 0.97). Bland-Altman difference plots had significant constant positive biases in the random compared with periphery and center methods. Mean differences ( $M_D$ ) and

confidence intervals ( $CI_{\text{mean}}$ ) were 0.11 ( $CI_{\text{mean}} = 0.06 - 0.16$ ) and 0.09 ( $CI_{\text{mean}} = 0.04 - 0.13$ ) for random versus periphery and random versus center, respectively. No significant bias was detected between periphery and center methods ( $MD(\text{periphery vs center}) = -0.02$ ;  $CI_{\text{mean}} = -0.06 - 0.01$ ).

**Conclusion:** Microscopic area for differential cell counting impacts neutrophil percentages with potential to affect cytologic diagnoses.

November 4, 2018

3:45 PM – 4:00 PM

## **EFFECTS OF MARKED HYPERTRIGLYCERIDEMIA AND LIPID CLEARANCE ON CANINE BIOCHEMISTRY TESTING**

Carolina Azevedo, Jonathan Lidbury, Unity Jeffery

**Background:** Triglyceride concentrations in dogs with hyperlipidemic disorders often exceed concentrations used for interference testing by biochemistry assay manufacturers. High-speed centrifugation or polar solvents (e.g. LipoClear) are often used to reduce triglyceride concentrations, but their efficacy requires evaluation in veterinary species.

**Objectives:** To determine the effect of marked hypertriglyceridemia on canine biochemistry testing; to assess the ability of high-speed centrifugation or LipoClear to correct lipemic interferences and to determine if LipoClear introduces inaccuracy into biochemistry assays.

**Methods:** Fifteen pooled canine serum samples were aliquoted and spiked with equal volumes of water or Intralipid (final concentration 3000 mg/dL). Intralipid aliquots underwent lipid removal by high-speed centrifugation or LipoClear, and a water-spiked aliquot underwent LipoClear treatment. Biochemistry panels were performed using a Vitros 4600 biochemistry analyzer. Results were compared by paired t-test or Wilcoxon test. Total observed errors were calculated between the water-spiked control and treated aliquots and considered acceptable if below the ASVCP guidelines.

**Results:** Statistically significant interferences were introduced by Intralipid for 16/18 analytes, with unacceptable errors for at least 4/15 pools for chloride, potassium, and enzymatic carbon dioxide. For these analytes, observed errors remained unacceptably high for at least 1/15 pools after centrifugation or LipoClear treatment. LipoClear treatment of a water-spiked sample introduced statistically significant differences for 10/15 analytes, with at least one pool exceeding acceptable error for 5/15 analytes.

**Conclusions:** Marked hypertriglyceridemia introduces clinically significant interferences for chloride, potassium, and enzymatic carbon dioxide, which were not identified in the manufacturer's literature. LipoClear introduces inaccuracy into biochemistry testing.

November 4, 2018

4:00 PM – 4:15 PM

## **HEMATOLOGIC AND BIOCHEMICAL PROFILES OF CATS NATURALLY INFECTED WITH CYTAUXZON FELIS, 29 CASES (2007-2017)**

Brandy Kastl, Lisa Pohlman, Nora Springer

**Background:** Reported hematological findings in *Cytauxzoon felis* are conflicting, incomplete, or limited by small sample size. With the exception of hyperbilirubinemia and icterus, the biochemical profile of cats with *C. felis* has not been described. Identification of common laboratory findings might assist practitioners in the diagnosis of *C. felis* when intraerythrocytic piroplasm identification is uncertain.

**Objective:** Retrospectively characterize hematologic and biochemical variables in a large cohort of cats naturally infected with *C. felis*.

**Methods:** Inclusion criteria were antemortem diagnosis of *C. felis* by identification of characteristic piroplasms in peripheral blood smears, a CBC, and biochemical profile.

**Results:** Most parasitemic cats were anemic (72.4%, 21/29) and nonregenerative (100%, 29/29), with icteric serum (93.1%, 27/29), and hyperbilirubinemia (96.6%, 28/29). Leukopenia (79.3%, 23/29) due to marked lymphopenia (96.5%, 28/29) and neutropenia (48.3%, 14/29) with left shift (44.8%, 13/29) was common. When reported, platelets were decreased in all cases (100%, 23/23). Schizonts were visible in 24.1% (7/29) of cases. Common biochemical changes included hypoproteinemia (72.4%, 21/29), hypoalbuminemia (93.1%, 27/29), hyperglycemia (79.3%, 23/29), decreased ALP (62.0%, 18/29), hyponatremia (82.8%, 24/29), hypochloremia (75.9%, 22/29), hypokalemia (58.6%, 17/29), hypocalcemia (51.7%, 15/29), and increased CK activity (51.7%, 15/29).

**Conclusions:** The index of suspicion for *Cytauxzoon felis* infection should be increased in icteric cats with nonregenerative anemia and concurrent leukopenia or thrombocytopenia. Decreased ALP activity is a unique and unexpected finding in icteric cats. The specificity of decreased ALP for cytauxzoonosis versus other common causes of icterus (hepatic lipidosis, cholangiohepatitis, or secondary hemolytic anemias) is currently being evaluated.

November 4, 2018

4:15 PM – 4:30 PM

## **HYPERCHOLESTEROLEMIA AND HYPERTRIGLYCERIDEMIA AS BIOMARKERS OF DISEASE IN COMPANION RABBITS**

Diya Sharma, Ashley Hill, Mary Christopher

**Background:** Inflammation has important effects on lipid metabolism, but the relationship between hyperlipidemia, inflammation, and disease remains unknown in rabbits. While rabbits are sensitive to dietary hypercholesterolemia, the etiology of hyperlipidemia when fed non-atherogenic diets is uncertain.

**Objectives:** The study's aims were to determine the association between hypercholesterolemia and patient characteristics, diseases, and select CBC and biochemistry analytes in rabbits, independent of diet, and to measure plasma lipoprotein lipid fractions in rabbits with inflammatory and other diseases.

**Methods:** CBC and plasma biochemistry data, including total cholesterol concentration, were retrospectively evaluated in 531 companion rabbits. Lipoprotein cholesterol fractions (non-HDLc and HDLc) and triglycerides were measured using a colorimetric enzymatic assay in archived plasma from a subset of 267 rabbits. Rabbits were categorized by age, sex, spay/neuter status, breed, diet status (fed atherogenic or non-atherogenic items), diseased organ system, and pathologic processes.

**Results:** Cholesterol was associated with fibrinogen ( $p = 0.01$ ), globulins ( $p < 0.01$ ), and heterophil ( $p < 0.01$ ) concentrations. Adjusting for diet, rabbits with severe infection or sepsis (OR = 13.25, 95% CI = 5.83–30.12), renal failure (OR = 14.42, 95% CI = 5.69–36.54) and hepatopathy (OR = 8.55, 95% CI = 3.55–20.62) had increased risk of hypercholesterolemia. Increased non-HDLc and triglyceride concentrations were also associated with these three disease states ( $p < 0.05$ ).

**Conclusions:** Independent of diet, hyperlipidemia is associated with biochemical and CBC markers of inflammation, and with severe infection or sepsis, renal failure, and hepatopathy. Increased total cholesterol, non-HDLc, and triglycerides are indicators of disease in companion rabbits.

## Clinical Pathology Focused Scientific Session II

November 6, 2018 | 8:00 AM – 12:00 PM

Session Chair: Julie Webb, DVM, DACVP, IDEXX Laboratories, Markham, Ontario

November 6, 2018

8:00 AM – 8:15 AM

### **A SPECIFIC IMMUNOASSAY AND ANTIBODY FOR DETECTION OF FELINE KIDNEY INJURY MOLECULE-1 IN URINE AND KIDNEY TISSUE**

Susan Bland, Mary Clark, Olivier Cote, Dorothee Bienzle

**Objectives:** Develop a urine point-of-care test for kidney injury molecule (KIM)-1 and evaluate it in healthy and diseased cats.

**Method:** Part of the feline KIM-1 gene was amplified, ligated into a plasmid, and transfected into a mammalian cell line. Supernatant was purified and tested for fusion protein by gel electrophoresis and Western blot. Mice were immunized with purified protein, and hybridomas were generated. Resulting monoclonal antibodies were tested by ELISA for detection of recombinant feline KIM-1 (rfKIM-1). A lateral flow assay (LFA) was constructed, and tested with 34 urine samples from healthy and diseased cats. Antibodies were also tested for reactivity with formalin-fixed paraffin-embedded kidney tissue.

**Results:** The LFA detected between 0.4 and 60 ng/mL of rfKIM-1 . Urine samples from healthy cats yielded optical density (OD) ratios of rfKIM-1 to naïve urine KIM-1 of 4.8 to 8.8. Samples from cats with suspected or confirmed acute kidney injury (AKI) had OD values ranging from 1.6 to 20.5. Urine KIM-1 varied over sequential days in cats with suspected AKI. In tissue sections, KIM-1 antibodies labeled tubular cells with morphological features of injury.

**Conclusions:** A rapid point-of-care assay for KIM-1 in feline urine has been developed. Preliminary results show marked increases in cats with disease-associated AKI, and expression in injured tubules. Initial results indicate that the LFA is sensitive and specific for KIM-1 in cats with AKI, values associated with different types of injury, duration of illness, different urine concentrations and prolonged storage remain to be investigated.

November 6, 2018

8:15 AM – 8:30 AM

### **VALIDATION OF THE USE OF DENSITOMETRIC QUANTITATION FOR MEASUREMENT OF SERUM M-PROTEIN CONCENTRATION IN THE DOG**

Robert Harris, A. Moore

**Background:** Densitometric quantitation of monoclonal protein (M-protein) using serum protein electrophoresis (SPE) is the standard method to monitor tumor burden of immunoglobulin secreting tumors in humans but has not been validated in the dog. Perpendicular drop, conservative perpendicular drop and tangential skimming methods have been proposed to derive percentage of M-protein from the SPE.

**Objectives:** Determine which method for quantitation of M-protein performs best in the dog. Characterize its performance using method validation experiments.

**Methods:** Serum from normal dogs or dogs with a gamma region IgG monoclonal gammopathy were used. Monoclonal IgG was purified and used in a spike and recovery experiment to determine the method producing the least bias. Method validation experiments to characterize interrater and intra-run variability, linearity under dilution and lower limit of detection were performed using this method and a biuret protein.

**Results:** Total allowable error was predetermined at 20%. Purified monoclonal IgG was produced. The conservative perpendicular drop method performed best with -17.9% bias at high (42.7% and 2.91 gm/dL) and 2.7% bias at low (4.7% and 0.72 gm/dL) M-protein levels. Linearity was deemed excellent for both M-protein percentage and concentration ( $r = 0.997$  and  $0.999$ , respectively). The lower limit of detection was 0.10 gm/dL and 2.20%. Intrarun and interrater CV for M-protein concentration was 1.1% and 3.2%, respectively; and for percentage of M-protein was 1.1% and 3.5%, respectively.

**Conclusion:** Densitometric quantitation is a valid technique for measuring IgG M-proteins in the gamma region using the conservative perpendicular drop and a biuret total protein.

November 6, 2018

8:30 AM – 8:45 AM

## **OPTIMIZING THE U411 AUTOMATED URINALYSIS INSTRUMENT FOR VETERINARY USE**

Samantha Evans, Julia Sharp, Linda Vap

**Background:** The Cobas u411 Analyzer (Roche Diagnostics, Indianapolis, IN) is an automated, reflectance photometry-based urinalysis instrument designed for use with Roche's CHEMSTRIP(R) 10UA technology and human urine samples.

**Objective:** Optimize and validate the Cobas u411 Analyzer for use in canine and feline urinalysis.

**Methods:** Patient urine samples presenting to the Clinical Pathology Laboratory at the Colorado State University Veterinary Teaching Hospital were analyzed with the Cobas u411 and by manual readings in parallel. Initially, 223 canine and 83 feline urine samples were run using the u411 factory settings. Following comparison with manual results and evaluation for directional bias, adjustments to the reflectance values were made in the instrument's programming. An additional 183 canine and 95 feline samples were run using the adjusted settings. Total protein concentration was measured in 48 samples and used to generate receiver operating characteristic (ROC) curves for the protein test pad.

**Results:** Following adjustments in reflectance programming, concordance between u411 and manual results was increased by 17.7% for protein, 11.7% for ketones, and 4.5% for bilirubin. Concordance for pH, glucose, and blood were not substantially changed. Discordance for all analytes was  $\leq 3\%$ . Canine and feline samples had similar

levels of discordance, though marginal concordance was higher for dogs with ketones, bilirubin, and blood.

**Conclusions:** Adjustments to the reflectance programming of the Cobas u411 Analyzer improved concordance with manual results for canine and feline samples. This instrument has the potential to greatly increase both efficiency and consistency of urinalysis procedures in high throughput veterinary diagnostic laboratories.

November 6, 2018

8:45 AM – 9:00 AM

### **THE ROLE OF HIGH-DENSITY LIPOPROTEINS (HDL) IN MODULATING NEUTROPHIL ACTIVATION DURING THE TRANSITION PERIOD IN COWS**

José Cruz Otero, Cheryl Wong, Erica Behling-Kelly

**Background:** The 3 weeks before and after calving, the transition period (TrPe), is a high-risk period for the development of costly production-related diseases in dairy cows. During the TrPe, changes in lipid metabolism are thought to contribute to disease risk. High-density lipoprotein (HDL) has anti-inflammatory activity in a number of species. We hypothesized that bovine HDL has immunomodulatory activity that is negatively impacted by the metabolic demands of the TrPe.

**Objectives:** Test the biological impact of bovine HDL isolated throughout TrPe on neutrophil activation.

**Methods:** HDL was isolated from EDTA-anticoagulated blood collected weekly throughout the TrPe from 12 adult multiparous Holsteins using density gradient ultracentrifugation. Heparinized blood from mid-lactation cows was treated for 4 hours with 50ng/ml LPS alone and in combination with 300mg/ml HDL. Dialysis buffer was the vehicle control. Neutrophil activation was determined by measuring changes in CD11b expression using flow cytometry.

**Results:** Pre-calving HDL decreased LPS-induced CD11b expression by 23% (SD  $\pm$  14.6). HDL from near-calving was pro-inflammatory and increased LPS-induced neutrophil activation of neutrophils by 45% (SD  $\pm$  233.9). HDL did not regain immunomodulatory activity post-calving in 6/12 cows.

**Conclusion:** Bovine HDL influences LPS-induced neutrophil activation in a manner that is dependent on the time relative to calving. The loss of HDL anti-inflammatory function near calving may contribute to the dysregulated inflammation that occurs during the TrPe *in-vivo*. Future studies are required to identify the HDL motif(s) involved in modulating LPS activity and develop interventions to optimize HDL's immunomodulatory activity through the TrPe.

November 6, 2018

10:30 AM – 10:45 AM

### **CLINICAL AND FLOW CYTOMETRIC FEATURES OF CANINE NODAL CD8+ AND CD4-CD8- T-CELL LYMPHOMAS**

Lauren Harris, Emily Rout, Janna Yoshimoto, Anne Avery

**Background:** Canine T-cell lymphoma (TCL) encompasses a heterogeneous group of diseases with variable clinical presentations, cytomorphologies, immunophenotypes, and biologic behaviors. The most common types of nodal TCL in dogs include indolent T-zone lymphoma (TZL) and biologically aggressive CD4+ peripheral T-cell lymphoma. Less common TCL phenotypes can be categorized by patterns in expression of surface antigen molecules, CD4 and CD8. The clinical and flow cytometry features of these less common nodal TCL subtypes, including CD8+ and CD4-CD8- TCLs, are not well characterized.

**Objective:** We aim to describe and correlate the clinical presentations, flow cytometry, and outcomes for a large cohort of nodal canine CD8+ and CD4-CD8- TCLs.

**Methods:** TCL cases were identified through the Colorado State University Clinical Immunology Laboratory (CSU-CI). Peripheral lymph node aspirates were identified as non-TZL TCL based on the expression of pan-leukocyte marker CD45 and T-cell subset markers CD3 and/or CD5. Cases with a CD8+ or CD4-CD8- phenotype were subsequently selected for further evaluation.

**Results:** Over 100 cases of CD8+ and CD4-CD8- TCLs met inclusion criteria. Approximately half of the patients with CD8+ TCL had cutaneous involvement, which was associated with prolonged overall survival compared to those with primary nodal disease. Cell size of CD8+ TCL also impacted overall survival. Expression of MHC class II, however, was not prognostically significant.

**Conclusions:** In this study we evaluate a large cohort of nodal CD8+ and CD4-CD8- TCLs and provide comprehensive analysis of the clinical and flow cytometric features of these poorly understood entities.

November 6, 2018

10:45 AM – 11:00 AM

## **CLINICAL DESCRIPTION AND LINEAGE ASSESSMENT OF CANINE CD34+ ACUTE LEUKEMIAS - IMMUNOPHENOTYPING AND GENE EXPRESSION PROFILING**

Emily Rout, Janna Yoshimoto, Julia Labadie, Anne Avery, Paul Avery

**Background:** Acute leukemias are aggressive neoplasms of immature hematopoietic cells, which express the stem cell marker CD34. Acute leukemias may arise from myeloid or lymphoid lineages, but it is often difficult to definitively determine lineage.

**Objectives:** To assess the clinical presentation, flow cytometry features, outcome, and gene expression of canine CD34+ acute leukemias, and determine whether flow cytometry is useful in lineage assignment.

**Methods:** We examined the immunophenotype of 192 canine cases of CD34+ class II MHC- acute leukemia. In a subset of cases, we examined patient characteristics, cytomorphology, clonality by PCR for antigen receptor rearrangements (PARR), and outcome. We used Nanostring technology to measure expression of selected myeloid, B-cell, and T-cell specific genes.

Three phenotypes were identified by flow cytometry: myelomonocytic (CD14+/MHCII-), T-lymphoid (CD5+) and undetermined (lineage-negative). Myelomonocytic and lymphoid cases had different leukocyte counts, breed distribution and age range. Similar to human CD34+ leukemias, a significant number of cases had clonally rearranged both immunoglobulin and T-cell receptor genes. T-lymphoid and undetermined-lineage cases frequently had a clonal T-cell receptor alone, but no cases had a clonal immunoglobulin receptor alone. Gene expression analysis confirmed increased expression of myelomonocytic genes (CD13, CD11c, CD11b, lysozyme, myeloperoxidase) and T-cell specific genes (CD3, TCRg, CD7, CD2, CD5) in CD14+/MHCII- and CD5+ cases respectively. Median overall survival was 20 days for all cases, with no difference among phenotypes.

**Conclusions:** Flow cytometry may help determine cell lineage in a subset of canine acute leukemias, which may facilitate the pursuit of targeted, lineage-specific therapies.

November 6, 2018

11:00 AM – 11:15 AM

**DIAGNOSTIC VALIDATION OF A WHOLE-SLIDE IMAGING SCANNER IN CYTOLOGICAL SAMPLES: DIAGNOSTIC ACCURACY AND COMPARISON WITH LIGHT MICROSCOPY**

Federico Bonsembiante, Ugo Bonfanti, Francesco Cian, Beatrice Zattoni, Laura Cavicchioli, Maria Elena Gelain

**Background:** Whole-slide imaging scanners allow the users to digitalize entire glass slides to obtain digital slides, which can be evaluated by clinical pathologists located in remote sites. A complete validation process is needed before this technology can be applied to routine cytological diagnostics. The aim of this study is to validate a whole-slide imaging scanner for cytological samples.

**Materials and methods:** Sixty cytological samples, whose diagnoses were confirmed by gold standard exams (histology or flow cytometry), were digitalized using a whole-slide imaging scanner to obtain digital slides. Digital slides and glass slides were examined by three observers with different levels of cytopathological expertise.

**Results:** No significant differences were noted between digital and glass slides as regard the number of cases correctly diagnosed as well as in sensitivity, specificity and diagnostic accuracy, irrespective from the observers' expertise. The agreements between the digital slides and the gold standard exams were moderate to substantial, while the agreements between the glass slides and the gold standard exams were substantial for all the three observers. The intra-observer agreements between digital and glass slides were substantial to almost perfect. The inter-observer agreements when evaluating digital slides were moderate between observers one and two and between observers one and three while it was substantial between observers two and three.

**Conclusions:** Our study demonstrates that the digital slides produced by the whole-slide imaging scanner are adequate to diagnose cytological samples and can be used by clinical pathologists with any level of expertise.

November 6, 2018

11:15 AM – 11:30 AM

### **A NOVEL AGGRESSIVE T-CELL LYMPHOMA/LEUKEMIA IN YOUNG ENGLISH BULLDOGS**

Kari Frankhouse, Emily Rout, Kelly Hughes, Janna Yoshimoto, Paul Avery, Anne Avery

**Background:** T-cell lymphoma/leukemia accounts for approximately 30% of all types of canine lymphoproliferative disease, with some subtypes demonstrating breed predilections. Through routine immunophenotyping of peripheral blood samples, we observed a unique form of T-cell lymphoma/leukemia, often presenting in young English bulldogs.

**Objectives:** To evaluate frequency, clinical presentation and outcome of this lymphoma/leukemia subtype.

**Methods:** The CSU Clinical Immunology database, containing approximately 7500 unique canine blood samples, was queried for the phenotype observed in young English bulldogs: CD4-/CD8-/class II MHC low T-cell lymphoma/leukemia. We reviewed available blood smears and gathered clinical information for English bulldog cases to assess presentation and outcome.

**Results:** A total of 118 cases of CD4-/CD8-/class II MHC low T-cell lymphoma/leukemia were identified from 2012-2018. 52 cases (44%) were English bulldogs. The median age among English bulldogs alone and non-English bulldog cases was 3.0 and 4.5 years, respectively (range 1-15 years). 65% of English bulldogs and 61% of non-English bulldog cases were male. Among English bulldog cases with available clinical information, 63% had splenomegaly, 46% had hepatomegaly, and 54% had gastrointestinal signs. 58% of cases had elevated liver enzymes, 75% were thrombocytopenic and 40% were anemic. Lymphocytes were small-sized with condensed chromatin, no nucleoli and scant basophilic cytoplasm. Among 36 cases treated with a range of chemotherapy protocols, the median survival was 18 days (range, 1-490 days).

**Conclusions:** CD4-/CD8-/class II MHC low T-cell lymphoma/leukemia is a rare aggressive T-cell disease, with a predilection for English bulldogs. Cases are young, predominantly male, and frequently have hepatic and splenic involvement.

November 6, 2018

11:30 AM – 11:45 AM

### **PREDICTION OF CANINE LYMPHOMAS' PHENOTYPE IN CYTOLOGICAL SAMPLES USING DEEP LEARNING**

Tommaso Banzato, Maria Gelain, Matteo Serraglio, Valeria Martini, Federico Bonsembiante

**Background:** Convolutional neural networks (CNN) are considered as the state-of-the-art image analysis and classification technique. In human medicine deep learning has achieved promising results in several different disciplines, but no reports about the possible applications in veterinary diagnostic pathology are currently available. In cytological classification of canine lymphoma, the possibility to define phenotype based on neoplastic lymphocytes morphology is still debated.

**Objective:** Predict the immunophenotype of canine lymphoma from cytological slides using deep learning.

**Methods:** Thirty-one lymphoma cases (20 B-cell and 11 T-Cell) with flow cytometry-confirmed immunophenotype were retrospectively selected. Digital whole-slide images were acquired. Photographs of a variable number of individual cells (minimum 250) were obtained for each case. Images were then saved into different folders according to the immunophenotype. The images were randomly divided into a training, a validation and a test set using a 70%, 15%, 15% scheme. A pre-trained CNN (Inception V3) was retrained and fine-tuned on our database until an 80% accuracy in the test set was achieved. Thereafter, the diagnostic accuracy of the fine-tuned CNN was tested using a leave-one-out cross-validation. The predicted immunophenotype for each case was assigned calculating the modal predicted immunophenotype of the images.

**Results:** The immunophenotype was correctly predicted in 30/31 cases and the area under the curve was 0.96 (95% confidence interval (CI): 0.82-1), the sensitivity was 100% (CI: 82.35-100.00), and the specificity was 91.67% (CI: 61.52-99.79).

**Conclusions:** Deep learning could potentially be used to predict the immunophenotype of lymphomas from cytological slides.

November 6, 2018

11:45 AM – 12:00 PM

## **IT IS TIME TO SIMPLIFY THE CLASSIFICATION OF CANINE AND FELINE BODY CAVITY EFFUSIONS**

Andrea Bohn

**Background:** There is occasional discussion regarding the traditional classification system for body cavity effusions in veterinary medicine. In particular, the category of modified transudate can be confusing and it is not clear how its parameters were established.

**Objective:** Retrospectively review a large number of cases to determine if the category of modified transudate is clinically useful or if a simpler classification scheme, using cutoff concentrations of 3,000 cells/ul and 2.5 g/dl protein to detect hypercellular samples and differentiate high vs. low protein transudation, respectively, will be at least as useful in developing differential lists and efficient diagnostic plans.

**Methods:** Medical records were evaluated from cats and dogs that were admitted to the veterinary teaching hospital at Colorado State University and had abdominal or thoracic effusion fluid evaluated by the clinical pathology laboratory. Data from fluid analyses

and cause, as determined by the medical record, were entered into an Excel spreadsheet. Traditional and simplified effusion classifications were evaluated and compared with respect to underlying causes.

**Results:** Evaluated were 440 canine and 152 feline abdominal effusions and 201 canine and 118 feline thoracic effusions. In nearly all cases where the traditional fluid analysis classification varied from the simpler classification system, the new system was a better match with the underlying cause of the effusion. The traditional classification scheme provided no additional diagnostic value.

**Conclusions:** A simpler method for classification of body cavity effusions is recommended. Knowing the more common causes of each type of effusion can help direct efficient diagnostic plans.

## Clinical Pathology Focused Scientific Poster Session

### C-02: POLYCLONAL B-CELL LYMPHOCYTOSIS IN A SUBSET OF ENGLISH BULLDOGS

Emily Rout, Julia Labadie, Robert Burnett, A Moore, Anne Avery

**Background:** A previous study of B-cell chronic lymphocytic leukemia (B-CLL) revealed that English bulldogs had a unique presentation, raising the possibility that English bulldogs have a novel form of B-CLL or a different B-cell lymphoproliferative disease. We have since observed that the expanded B-cell population in these English bulldogs is often polyclonal, while the vast majority of non-English bulldogs with B-CLL are clonal.

**Objectives:** Examine cases of B-cell lymphocytosis in English bulldogs, assessing clinical presentation, clonality and outcome.

**Methods:** We queried the CSU Clinical Immunology database for English bulldog cases from 2010-2018, with  $>5,000$  lymphocytes/ul in the blood and  $>60\%$  small CD21+ lymphocytes by flow cytometry. PCR for antigen receptor rearrangements (PARR) was performed in 45 cases, and a smaller subset had additional PARR analysis, targeting additional heavy chain genes, kappa deleting element, and lambda light chain genes. Clinical information was gathered for a subset of cases.

**Results:** 73 cases met the diagnostic criteria. The median age was 7.3 years (range 2.0-13.5 years). There were more males (71%) than females (29%). The median lymphocyte count was 26,000/ul (range 5,000-283,836/ul). Splenomegaly, hyperglobulinemia and anemia were common, while lymphadenopathy and thrombocytopenia were rare. PARR analysis revealed only 38% (17/45) of cases had clonal immunoglobulin genes. Outcome in 11 cases suggests an indolent clinical course (median survival time not reached).

**Conclusions:** A subset of English bulldogs, particularly young males, appear to develop persistent polyclonal B-cell lymphocytosis, with an indolent course. We hypothesize this is a non-neoplastic process and may have a genetic basis.

### C-03: A RETROSPECTIVE STUDY OF LYMPHOCYTE-RICH EFFUSION IN BRAZILIAN CATS

Stella Valle, Naila Duda, Nilson Nunes, Daiani Wissmann, Bruno Almeida, Thaianie Oliveira, Angelica Menin, Fernanda Costa

**Background:** Lymphocyte-rich transudate and exudate can be associated with several pathophysiology mechanism in cats.

**Objectives:** To investigate the characteristics of lymphocytic abdominal and pleural effusion in cats.

**Methods:** For the retrospective study, 25 lymphocytic pleural and abdominal feline effusion were selected from the laboratory database, between 2015 and 2018. Physical

(color, aspect), chemical (glucose, TP by biuret method) and cytologic parameters were obtained from all samples. The TNCC was determined by automated cell-counter and the squash smears were obtained from centrifuged EDTA samples. Romanowsky stained squash smears were used to differentiate 200 cells, counted by the same operator. Additionally, chemical tests were performed as necessary.

**Results:** Out of 25 samples, 2 were from abdominal cavity. Three cats had a FIV/FeLV negative test. The appearance was red and slightly turbid in 48% (n= 12), orange and turbid in 44% (n= 11) and 2 samples had milky aspect high concentration of triglycerides. In a chemical evaluation, the TP concentration was high in 4 samples (>5 g/dL) and the glucose was low (< 50 mg/dL) in 10 (40%). Excluding the chylous effusion samples, the TNCC mean was 39.920/mL. In the cytology evaluation, the majority of pleural effusion (n= 15, 60%) presented a predominance of large and medium dysplastic lymphocytes, a chylothorax included. One cat, negative to FIV/FeLV, had a thyroid ectopic carcinoma, histopathologically diagnosed.

**Conclusion:** The pleural effusion was the most common and lymphoma was likely to be the underlying pathophysiology of the fluid formation and can also be associated with FeLV positivity.

#### **C-04: BIOCHEMISTRY ANALYSIS OF POST-MORTEM VITREOUS HUMOR AND URINE SAMPLES FROM BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) FOR LOW SALINITY EXPOSURE**

Ruth Ewing, Adam Schaefer, Debra Moore, Ruth Carmichael, Heidi Whitehead, Tracy Stokol

**Background:** Hyponatremia, hypochloremia, and hypo-osmolality, from environmental uptake, has previously been described from serum samples collected from living coastal dolphins exposed to hyposalinity. Post-mortem serum is not diagnostically suitable for biochemical analysis; therefore, vitreous humor and urine were collected for evaluation.

**Objective:** We hypothesized that sodium (Na) and chloride (Cl) concentrations and osmolality in vitreous humor and urine would be decreased in dead dolphins exposed to freshwater.

**Method:** Samples were collected from bottlenose dolphins found dead in low salinity (<10 ppt) or with freshwater associated skin lesions (D-fresh) and from bottlenose dolphins and pelagic species (various whales) found dead in normal salinity (>20 ppt, DP-saline). Sodium, chloride, urea nitrogen (UN) and creatinine concentrations were measured with an automated chemistry analyzer and osmolality was measured by freezing point depression.

**Results:** Preliminary analysis demonstrated median electrolyte concentrations were lower in vitreous and urine in D-fresh versus DP-saline animals, with less apparent changes in osmolality. Median vitreous UN and urine creatinine concentrations were higher and lower in D-fresh versus DP-saline, respectively. Only two D-fresh animals had NaCl concentrations within the range of DP-saline animals, suggesting these animals were transitioning and possibly osmoregulating between low and high salinity

exposure. Additionally, one whale in normal salinity had low NaCl concentrations similar to D-fresh animals.

**Conclusion:** Our preliminary results support the use of vitreous humor and urine electrolyte concentrations for identification of freshwater exposure in dolphins found dead. Additional samples from dolphins under low and normal salinity conditions are needed for diagnostic application.

#### **C-05: CLINICOPATHOLOGICAL FINDINGS IN A CHRONIC KIDNEY DISEASE DOG TREATED WITH MESENCHYMAL STEM CELLS**

Ellen Marquez, Livia Bestana, Rafael Castilho, Giovani Araújo

The kidney has an important role by removing waste products and regulating metabolic functions, and Chronic Kidney Disease (CKD) creates substantial problems that can result in the death of the patient. Classic treatment for CKD includes fluid therapy, diuretics, antioxidants, antibiotics for infection, gastroprotectants, and anti-nausea agents. Mesenchymal Stem Cells (MSC) have been suggested as an alternative treatment of different diseases. Studies in animal models with CKD have demonstrated the potential of these cells for improving function and regeneration of a damaged kidney. The focus of this study is to report the hematological, biochemical and urinalysis changes in a dog with advanced CKD submitted to the regenerative therapy. A 10-years-old, castrated male, Pitbull dog was presented in the Veterinary Hospital with clinical signs of vomiting, anorexia and apathy. Blood samples for the hemogram and biochemical profile were required. Urine was taken for a urinalysis and protein/creatinine (P/C) ratio determination. Since the subject dog has a previous history of CKD and out of range test results with conventional treatments, it was suggested to consider the MSC therapy. The previous and subsequent hemogram parameters revealed results between of the reference values. In contrast, the previous biochemical and urinalysis tests showed a renal azotemia, hypocalcemia, hyperphosphatemia, and hypokalemia, with a low specific gravity and proteinuria. The P/C ratio revealed normal. All the parameters returned to the reference values after administration of MSC, indicating the importance of clinico pathology data on monitoring the regenerative potential of MSC to provide functional recovery from CKD dogs.

#### **C-06: LABORATORY EVALUATIONS IN A DOG WITH CHOLANGITIS/CHOLANGIOHEPATITIS SUBMITTED TO STEM CELLS THERAPY**

Ellen Marquez, Livia Bestana, Kalinca Gromboni

A 6-year-old female dog was admitted to the Veterinary Hospital Araújo with icterus, apathy, weight loss and dark urine. The laboratory findings were icteric plasma, increased of ATL, total, direct and indirect bilirubin, alkaline phosphatase and GGT. The exams also revealed a decrease in glucose and urea. Ten days after the first appointment, the dog came back with the same symptoms and the veterinary administered injectable dexamethasone and erythropoietin, prescribed ursodeoxycholic acid, ademetonine, metronidazole, plasil, enrofloxacin, a vitamin complex for the liver, and scopolamine butylbromide. They repeated the exams and the animal presented hypochromic normocytic anemia, anisocytosis and polychromasia. The leukogram

showed a leukocytosis with neutrophilia and a mild regenerative left shift, monocytosis and lymphocytosis. In addition to that, also revealed a high GGT, low creatinine and intense jaundice. The diagnosis was cholangiohepatitis and the application of stem cells was performed. Five days after the stem cell therapy, the dog was with a better clinical status and the laboratorial exams had an improvement, there was no more anemia, the creatinine was normal and the jaundice recovered. In addition to treatment with stem cell ursodeoxycholic acid was recommended to the patient for chronic use. The dog had a clinical and laboratory evidence of improvement with the mesenchymal stem cell treatment.

#### **C-07: CONCURRENT ALIMENTARY PLASMACYTOMA AND DIFFUSE LARGE B-CELL LYMPHOMA IN A DOG**

Matthew Schexnayder, Matti Kiupel, Joshua Sparago, Stephanie Rutherford, Amy Grooters, Daniel Paulsen, Shannon Dehghanpir

An 11-year-old female spayed terrier mix presented with a chronic, intermittent history of blood-tinged regurgitation. An upper gastrointestinal endoscopic evaluation revealed an obstructive distal esophageal mass, as well as two gastric masses, located at the gastric fundus and pylorus. The cytopathologic interpretation of an impression smear of an endoscopically-obtained biopsy of the esophageal mass was plasma cell neoplasm, likely an extramedullary plasmacytoma, based on the presence of moderately well-differentiated plasma cells. Histopathologically, the esophageal mass consisted of many plasmacytoid cells, while the two gastric masses consisted of neoplastic large lymphocytes. In order to further characterize these masses as separate or associated lesions, immunohistochemistry and polymerase chain reaction for antigen receptor rearrangements (PARR) were performed. Based on clonal rearrangement of the immunoglobulin heavy gene and positivity for CD20 and MUM-1, the gastric masses were consistent with diffuse large B-cell lymphoma (DLBCL). The findings of negative PARR testing and CD20 staining with MUM-1 positivity supported a diagnosis of an extramedullary plasmacytoma of the esophageal mass. In people, DLBCL is rarely associated with Epstein-Barr virus (EBV; human herpesvirus 4), and a case of EBV-induced transformation of cutaneous plasmacytoma into DLBCL has been documented. To evaluate for an oncogenic gammaherpesvirus in this case, detection of herpesvirus (generic) by polymerase chain reaction was performed on both neoplasms, and the results were negative. This is the first report of a dog with esophageal extramedullary plasmacytoma and a second lymphoid neoplasm of gastric DLBCL.

#### **C-08: CYTOLOGIC IDENTIFICATION OF BACTERIA IN SPLENIC ASPIRATES FROM TWO MACAWS WITH COXIELLOSIS**

Kellie Whipple, Mary Leissinger, Justin Rosenberg, Leslie Charles, Lisa Farina, James Wellehan

Two female blue and gold macaws (*Ara ararauna*) from the same breeding collection presented eleven months apart for similar signs of weight loss despite adequate appetite, lethargy, and sitting at the bottom of the cage. Physical examinations of both revealed emaciation and reduced wing range of motion bilaterally. Splenomegaly was identified on radiographs. Splenic cytology in each case revealed a reactive lymphoid

population admixed with macrophages, heterophils, and myeloid precursors. Bacilli of varying length were found extracellularly and within vacuoles inside macrophages. In both cases, doxycycline therapy was recommended; the bird in case 1 died prior to initiation of therapy and the bird of case 2 was euthanized before therapy due to severity of clinical signs and possible infectious and/or zoonotic implications to the breeding collection. The main necropsy lesions in both birds were similar, with histiocytic to histiocytic and lymphoplasmacytic splenitis, hepatitis, and encephalitis. Organisms observed microscopically were identified as *Coxiella* sp. via 16S PCR and DNA sequencing of splenic aspirates. Avian coxiellosis is a rare, but increasingly reported disease, with reports in 11 psittacines of 8 different species plus a single toucan. Clinical information is not well described, as coxiellosis has been a post-mortem diagnosis in the majority of cases. It is currently unknown whether this disease is transmitted directly or through arthropod vectors. Zoonotic potential has not been elucidated, but is a concern since a similar organism (*Coxiella burnettii*) is the causative agent of the zoonosis Q fever.

#### **C-09: CYTOLOGICAL FEATURES OF A CANINE PITUITARY ADENOMA REMOVED BY TRANSSPHEOIDAL HYPOPHYSECTOMY**

Ilaria Cerchiaro, Jessica Chavera, Tina Owen, Annie Chen, Linda Martin, Joshua Ramsay, Margaret Miller, Jane Wardrop, Cleverson Souza

**Background:** In the canine species, pituitary masses are classified into adenomas, invasive adenomas and adenocarcinomas. Adenomas are the most common pituitary tumors and the type of pituitary neoplasms most frequently associated with pituitary-dependent hyperadrenocorticism (PDH).

**Objective:** Description of the cytological features of a canine pituitary adenoma surgically removed by transsphenoidal hypophysectomy.

**Methods:** A 7-year old female neutered Golden Retriever Mix was referred to the WSU Neurology Service as a potential candidate for transsphenoidal hypophysectomy after MR imaging revealed a mass most consistent with a pituitary macroadenoma. PDH was supported by clinical signs, serum biochemistry, urinalysis, ACTH stimulation test and abdominal ultrasound results.

**Results:** An impression cytology showed round to ovoid cells, with 1-5 variably sized nucleoli and a lightly basophilic, occasionally mildly vacuolated cytoplasm. Bi and trinucleation, satellite nuclei, nuclear molding and rare atypical mitotic figures were seen along with mild to moderate, occasionally marked anisocytosis and anisokaryosis. Highly foamy/vacuolated macrophages, occasionally displaying intracytoplasmic erythrocytes or hematoidin crystals and/or leukocytes, and bundles of spindle cells, occasionally associated with a moderate amount of an amorphous, eosinophilic material, were also present. A high number of free nuclei and rare lakes of a dense, purple, amorphous material were observed on the background. Histopathology and reticulin stain supported the cytologic diagnosis of pituitary adenoma. ACTH and MSH expression suggested a melanotroph type. Ki-67 proliferation index was high at 7.73%.

**Conclusions:** Intraoperative cytology can be useful for a preliminary assessment of a pituitary or sellar mass before histopathologic confirmation.

#### **C-10: CYTOLOGICAL FEATURES OF AN EXTRADURAL SPINAL SYNOVIAL CYST IN A DOG**

Ilaria Cerchiaro, Jessica Chavera, Hillary Greatting, Alisha Massa, Jane Wardrop, Cleverson Souza

**Background:** Extradural spinal synovial cysts are uncommon thoracolumbar spinal cord compressive lesions in dogs associated with degeneration of the articular facets and adjacent intervertebral disc.

**Objective:** Description of the cytological features of a canine extradural spinal synovial cyst.

**Methods:** A 10-year old, male neutered English Setter was presented to the WSU Neurology Service for evaluation of progressive weakness and incoordination of the rear limbs of one-month duration. The neurological examination demonstrated ambulatory thoracolumbar myelopathy. CSF tap from the lumbosacral cistern showed the nonspecific finding of a mild albuminocytologic dissociation. MRI of the thoracolumbar spine displayed an extradural compressive lesion in the right dorsolateral spinal canal at the level of the L1-L2 intervertebral disc space. The radiologic finding was considered most consistent with a synovial cyst, however, without further diagnostics a neoplastic process could not be completely excluded. Decompressive surgery confirmed the presence of an extradural compressive material associated with the articular facet which was submitted for microscopic evaluation.

**Results:** Impression cytology of the compressive lesion showed variably vacuolated, frequently granulated, ovoid to spindloid cells, believed to be synoviocytes. Also present were variably vacuolated macrophages, adipocytes, erythrocytes in a linear arrangement and an amorphous, eosinophilic material. The histopathology revealed a degenerative process of the articular facets and adjacent intervertebral disc supporting the clinical and radiologic diagnosis of extradural spinal synovial cyst.

**Conclusions:** Intraoperative cytology can aid in the diagnosis of an extradural spinal synovial cyst, and can help to exclude other conditions as cause for a progressive thoracolumbar myelopathy.

#### **C-11: DIFFERENTIATION OF NEOPLASIA FROM REACTIVE MESOTHELIUM IN CAVITY FLUID AND TUMOR ASPIRATE PREPARATIONS USING MULTIPLEX IMMUNOCYTOCHEMISTRY FOR VIMENTIN AND CYTOKERATIN**

Devin von Stade, A Moore, Kelly Santangelo, Sarah Leavell

**Background:** Multiplex immunocytochemistry (mICC) for a panel of cytokeratin and vimentin has been reported for use in veterinary species as a means of quickly characterizing expression of cytokeratin only (epithelial origin), vimentin only (mesenchymal origin) or co-expression (typically mesothelium) of these intermediate

filaments. When combined with morphologic appearance of the cytologic preparation, it can aid in the diagnosis of neoplasia.

**Objective:** Characterize the agreement of mICC for cytokeratin and vimentin and resultant cytologic interpretation with histologic findings.

**Methods:** Archived cytology cases with concurrent cytokeratin and vimentin mICC and biopsy or necropsy with histology of the tissue of clinical interest (n=19) were collected. Medical records, clinical follow-up, and results of the initial pathologic evaluations were collected and reviewed. Agreement of mICC findings with histopathology was determined and the observed sensitivity and specificity for a diagnosis of neoplasia versus non-neoplasia (inflammatory or reactive mesothelium) was calculated.

**Results:** 19 cases from dogs (n=17), 1 cat, and 1 guinea pig met inclusion criteria and included peritoneal (n=6), pericardial (n=1), and/or thoracic (n=9) fluid samples, and solid tumor (n=3) aspirates. They were categorized as carcinomas (n=7), sarcomas (n=5), or non-neoplastic (inflammatory/reactive) conditions (n=7) based on histopathology. Histopathology corroborated mICC findings in every case. When mICC findings were interpreted with the cytologic picture, there was discord between cytologic and histologic diagnosis in 2 cases of sarcoma, producing an observed 83.3% sensitivity and 100% specificity for detection of neoplasia.

**Conclusions:** Multiplex ICC is a useful adjunct to conventional cytology, having a strong association with histologic diagnosis.

#### **C-12: EFFECT OF URINE COLLECTION METHOD ON THE SENSITIVITY AND SPECIFICITY OF THE SEDIVUE DX(TM) ANALYZER FOR DETECTION OF CELLS AND CRYSTALS.**

Lydia Pena, Annalisa Hernandez, Johanna Heseltine, Mary Nabity

**Background:** The IDEXX SediVue Dx(TM) Urine Sediment Analyzer (SDx) performs automated urine analysis on samples from veterinary patients. Debris is commonly seen in voided samples and can interfere with accurate detection of formed elements.

**Objective:** To compare the sensitivity and specificity of the SDx, using manual microscopy as the reference method, for detecting cells and crystals in voided versus cystocentesis samples.

**Methods:** 158 urine samples with known collection method (72% cystocentesis, 28% voided) were evaluated for cells and crystals using the SDx (1.0.1.3) and manual microscopy. For the SDx, the reported results were used. For manual microscopy, sediment was prepared using a KOVA system, and each element was quantified by averaging the number of cells in 10 high power fields (HPF, 40x). For the purpose of sensitivity and specificity determination, a positive result was considered >5 cells/HPF or >1 crystal/HPF. 95% confidence intervals (CI) were calculated.

**Results:** On manual microscopy, 36%, 21%, 1%, 8%, 4%, and 2% of samples were positive for red blood cells, white blood cells, squamous epithelial cells, non-squamous

epithelial cells (NEC), struvite crystals, and calcium oxalate dihydrate crystals, respectively. For NEC, the specificity of the SDx was higher for cystocentesis samples (96%; CI: 90%-99%) than voided samples (73%; CI: 57%-86%). No difference was observed for the other elements.

**Conclusions:** For most elements evaluated, sample collection method did not appear to significantly impact their detection by the SDx. Further evaluation of collection method using more samples and including casts and bacteria is needed.

### **C-13: EPIDEMIOLOGICAL PROFILE OF DOGS WITH CAVITARY EFFUSION IN BRAZIL**

Flavio Alonso, Paulo Ricardo Paes

**Background:** Cavitory effusion (CE) represents a frequent clinical entity in the medical routine of dogs generally presenting with significant prognostic importance. Little information has been published regarding the profile of affected patients and about the laboratorial diagnostic accuracy of intracavitary neoplasia (IN).

**Objectives:** This study aimed to raise and enrich these data within a Brazilian socio-geographical context.

**Methods:** A retrospective study of CE cases was conducted to retrieve clinical, epidemiological and laboratory data from dogs consecutively consulted at the Veterinary Hospital of Universidade Federal de Minas Gerais (Brazil) between 2012 and 2016. 245 individuals were divided into groups according to their age, gender, breed, body size, effusion classification and type of underlying etiology. IN cases were divided in those diagnosed by body fluid cytology and histopathology.

**Results:** 60.4% (n=148) of animals were female, 27.7% (n=68) were large-sized (> 62 lbs), 10.6% (n=26) were Poodle and 56% (n=137) were elderly (> 7 y/o). Considering the underlying etiology, 52.9% of pleural effusions were neoplastic, 60.1% of peritoneal effusions were associated with severe hypoproteinemia (SHP), hypoalbuminemia and/or hepatopathy and 81.8% of pericardial effusions were exudates. The general sensitivity obtained for the diagnosis of neoplastic effusion by EC cytology was equal to 51.5% (ranging from 7 to 100%, according to tissue origin).

**Conclusions:** CE is a frequent manifestation in female, elderly, large-sized dogs, often caused by neoplasia, SHP or hepatopathy. Cytological sensitivity for IN diagnosis is greater for discrete round cells and lower for mesenchymal spindle cell origin.

### **C-14: GENERAL CLASSIFICATION OF CANINE CAVITARY EFFUSIONS BASED ON BIOCHEMICAL PARAMETERS**

Flavio Alonso, Paulo Ricardo Paes

**Background:** Distinction between transudates and exudates in human cavitory effusions (CE) currently involves the measurement and interpretation of multiple biochemical parameters with high analytical accuracy, while veterinary medicine is still

guided mainly by effusion total protein content (TPe) and total nucleated cell count (TNCCe) (traditional general system).

**Objectives:** To determine a new model based on biochemical parameters for general classification of canine CE, and thus, improve the identification of its underlying etiology.

**Methods:** Clinical, laboratorial and imaging data from 129 canine cases of peritoneal and pleural effusion were organized and submitted to multiple logistic regression modeling. Six different general classification ranges, based on TPe and TNCCe, were selected from literature and a gold standard based on clinical-pathological criteria was created.

**Results:** The best general classification range (TPe  $\geq$  2.0 g/dL and TNCC  $>$  5,000 cells/ $\mu$ L) showed an accuracy (73.6%) lower than biochemical parameters, such as ascitic cholesterol (COLa  $>$  41.2 mg/dL, accuracy = 88.7%, n=106) and pleural albumin (ALBp  $>$  0.8 g/dL, accuracy = 100%, n=112) for the identification of exudates. The best multiple predictive model for any cavity involved the variables glucose, albumin and TNCCe (n=66) and presented values of accuracy, sensitivity and specificity for the diagnosis of exudates equals to 94%, 96% and 90%, respectively.

**Conclusions:** The general biochemical classification of canine CE's has a higher diagnostic value compared to the simple traditional system and should be implemented in the clinical routine of canine patients.

#### **C-15: EVALUATION OF THROMBOELASTOGRAPHY IN CATS DURING THE DEVELOPMENT OF OBESITY AND WITH PROLONGED OBESITY**

Taylor Towns, Elizabeth Spangler, Robert Judd, Emily Graff

**Background:** In humans, obesity is associated with hypercoagulability leading to an increased risk of thromboembolism. Thromboelastography (TEG) is a useful tool in the assessment of hypercoagulability in multiple disease states of several mammalian species. A recent study of client-owned cats determined that obese cats have a significantly shortened R time compared to lean cats, suggesting hypercoagulability.

**Objective:** To evaluate changes in TEG parameters during the development and following prolonged obesity in a feline colony.

**Methods:** Eight lean, adult, purpose-bred, neutered male cats were fed an *ad-lib* chow diet to induce obesity, which developed over 18 months and was sustained for 48 months. Cats were weighed weekly and data including CBC, biochemistry profile and TEG were evaluated at baseline (prior to *ad lib* feeding), 18 months (development of obesity), and at 48 months (prolonged obesity).

**Results:** Following 18 months of *ad lib* feeding, body weight increased from  $3.8 \pm 0.29$  kg to  $5.9 \pm 0.73$  kg, with ~60% increase in body weight, which was maintained over 48 months. The mean TEG values at baseline were an R value of  $5.83 \pm 1.26$  seconds, K of  $1.70 \pm 0.498$  seconds, angle of  $66.84 \pm 6.13$  degrees and MA of  $54.8 \pm 6.68$  mm, consistent with normal coagulation status.

**Conclusion:** There were no significant changes from baseline for any value at 18 or 48 months. In contrast to the previous findings, we saw no significant differences in TEG parameters during the development of, or following prolonged obesity in our feline model.

#### **C-16: HEMATOLOGIC PARAMETERS AND THEIR CORRELATION WITH VIRAL AND PROVIRAL LOADS IN FELV NATURALLY INFECTED CATS**

Naila Duda, Lucía Ortiz, Ana Paula Murtele, Nilson Nunes, Fernanda Costa, Paulo Roehe, Félix González, Stella Valle

**Background:** FeLV positive cats with progressive and regressive infection can show hematologic changes due to bone marrow suppression.

**Objectives:** To correlate viral and proviral loads with blood and bone marrow parameters in FeLV naturally infected cats.

**Methods:** 34 cats were allocated in three groups based on clinical and hematologic parameters, results of FeLV ELISA test and viral/proviral FeLV loads in blood and bone marrow as follows: symptomatic progressive (high viral/proviral loads with clinical signs, n= 12), asymptomatic progressive (high viral/proviral loads without clinical signs, n= 9) and regressive infection (low viral/proviral loads with/without clinical signs, n= 13). CBC and bone marrow cytology were performed in all cats. Viral/proviral FeLV loads in blood and bone marrow was performed by qPCR. Hematological variables were compared among groups.

**Results:** Out of 34 cats, 13 were negative to ELISA test and showed low proviral loads in the bone marrow and categorized as regressive infection. Infected cats showed thrombocytopenia, lymphopenia, and non-regenerative anemia as the most prevalent clinical signs. Between groups, the erythroid parameters were significantly different in the symptomatic progressive group. In the bone marrow cytology MDS-Er, IMHA, ALL or lymphoma, AML-M6 and hypereosinophilic syndrome were observed in the symptomatic progressive and regressive. Comparing hematologic parameters and viral/proviral loads, significant correlation was observed in erythroid parameters only in asymptomatic progressive.

**Conclusion:** The viral/proviral loads are not responsible for the hematological signs observed in FeLV natural infection suggesting that the integration of the proviral DNA in the hematopoietic cells are responsible for the hematological alterations.

#### **C-17: HEMATOLOGICAL EVALUATION OF ALOUATTA BELZEBUL: MANUAL AND AUTOMATED METHODS**

Regina Takahira, Victor Guimaraes

**Background:** Red handed howler monkey (*Alouatta belzebul* Linnaeus, 1766) is an endemic species from Brazil and is classified as "Vulnerable" according to the International Union for Conservation of Nature (IUCN). Studies with this endangered

primate species addressing clinical and pathological features are either incipient or non-existent.

**Objective:** This study aimed to compare manual and automated hematological methods for *Alouatta belzebul* from the Lower Xingu River region (Para State, Brazil).

**Methods:** Blood samples were obtained from anesthetized animals by venipuncture of the femoral vein into tubes containing 5% EDTA. An aliquot of blood was submitted to manual analysis with the aid of hemocytometer and refractometer, and the other one was analyzed by impedance equipment (*ABX Micros 60*, Horiba®, California, USA).

**Results:** The mean value of erythrocyte counts by the automated method ( $4.22 \times 10^6/\mu\text{L}$ ) was significantly ( $p < 0.05$ ) higher than the manual method ( $3.27 \times 10^6/\mu\text{L}$ ) and the automated platelet count was significantly lower ( $88.69 \times 10^3/\mu\text{L}$ ) compared to manual counting ( $151.77 \times 10^3/\mu\text{L}$ ). The hematocrit (%) and total leukocyte count ( $\times 10^3/\mu\text{L}$ ) showed no statistical difference between the two methods. The manual determination of the hematimetric indices, global leucometry and platelet counts ensures greater distinctiveness of the size of the globules and data were actually consistent with the species. Such cell counter calibrated for human blood samples overestimated total erythrocytes counts and underestimated primate platelet counts.

**Conclusions:** The statistical difference found reinforces the expectations of the studies that mention considerable differences in the size of red blood cells and platelets among the species.

#### **C-18: IMPROVING THE CITOPATHOLOGICAL DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN LYMPH NODES SAMPLES**

Juliana Guerra, Natália Fernandes, Rodrigo Réssio, Jéssica Magno, Lidia Kimura, José Eduardo Barbosa, Helena Taniguchi, Roberto Hiramoto, Gabriela Motoie, José Eduardo Tolezano, Bruno Cogliati

**Background:** The identification of the parasite in cytological smears of lymph nodes aspirates is a widely applied technique for the direct diagnosis of canine visceral leishmaniasis (CVL). Although extremely specific, this method presents limited sensitivity and enhancing strategies is highly desirable.

**Objective:** To evaluate the diagnostic efficacy of smear cytology (SC), liquid-based cytology (LBC), cell block (CB) hematoxylin and eosin (H&E) and immunocytochemistry (ICC) of lymphoid samples for CVL diagnosis, by blinded comparisons, using serology and polymerase chain reaction (PCR) as the reference standard.

**Methods:** A sectional study with a convenience sample of 60 dogs, fifty dogs with CVL (serology and PCR) and ten negative controls, was carried out. Fine needle biopsy aspiration of popliteal lymph node was submitted to SC, LBC and CB-H&E and CB-ICC.

**Results:** The use of a preservative medium and centrifugation for cytological samples reduced the number of unsatisfactory cases due to low cellularity and excessive blood contamination. The specificity and predictive positive value were similar among all

methods. LBC sensitivity was 20.0% and CB-H&E was 22.0%. Otherwise, CB-ICC demonstrated significantly higher sensitivity (70.0%), compared to the SC alone (34.0%). Also, CB-ICC showed to be more effective in the detection of infected animals with mild clinical signs (71.8%).

**Conclusions:** LBC allowed an excellent cellular preservation and the application of ancillary techniques. CB-ICC is a promising tool to improve the cytopathological diagnosis of CVL and may be applied in routine epidemiological screening.

### **C-19: MARKED PARANEOPLASTIC EOSINOPHILIA IN A CAT WITH T-CELL LYMPHOMA**

Natalia Strandberg, Andrea Santos, Joanne Messick, Ashley Leisering, Yava Jones-Hall, Andrea Vanderpool

**Background:** A 2-year-old male neutered domestic shorthair cat was presented for anorexia, vomiting, and loose stools. Baseline data revealed a marked leukocytosis (73,800 cells/ $\mu$ L) due to a mature neutrophilia (22,900 / $\mu$ L) and extreme eosinophilia (38,390 / $\mu$ L) with less dramatic increases in mature lymphocytes (11,100 / $\mu$ L) and basophils (740 / $\mu$ L). A diffusely thickened jejunum, multiple nodules in spleen and colon, and a mesenteric lymphadenopathy were identified. Cytology of a splenic nodule and mesenteric lymph node revealed neutrophilic and eosinophilic inflammation/infiltration with bone marrow biopsy confirming a hypercellular process associated with a prominent proliferation of eosinophils. The maturation of the eosinophilic lineage was left shifted and morphologic features included abnormal granulation, cytoplasmic vacuoles, unusually large and bi-nucleated forms and asynchronous maturation. Despite empirical therapy, the extreme eosinophilia persisted and both immature and dysplastic forms appeared in the peripheral circulation. The cat's clinical signs waxed and waned over the following month, however his condition deteriorated soon thereafter and euthanasia was elected. At necropsy, a T-cell lymphoma (CD3+, CD 20-) with heavy eosinophilic infiltrates in various tissues and full thickness ulceration in the colon were diagnosed. PARR, other molecular studies, and IHC are pending to further characterize the neoplastic lymphoid population in this case.

**Purpose:** To describe an uncommon paraneoplastic response associated with T-cell lymphoma in a cat and highlight the difficulties in differentiating it from other causes of eosinophilia.

**Conclusions:** We hypothesize that the T-cell lymphoma was responsible for the cat's blood and tissue eosinophilia, leading to clinical manifestations.

### **C-20: METARUBRICYTES IN ASCITIC FLUID: A CYTOLOGICAL FINDING INDICATIVE OF INTRACAVITARY HEMANGIOSARCOMA?**

Paulo Ricardo Paes, Flavio Alonso

**Background:** Hemangiosarcoma (HS) is a neoplasm that frequently develops in the liver or spleen of dogs, causing cavitory effusion (CE) and is eventually associated with extramedullary hematopoiesis (EMH). Its cytological diagnosis, whether from FNAB or a

body fluid (BF) sample, is considered limited and usually requires histopathology for detection. EMH is cytologically characterized by the observation of hematopoietic precursors, among them, metarubricytes.

**Objective:** We speculate that, in cases where splenic or hepatic HS develops in association with EMH, the established CE could contain these cells.

**Methods:** BF samples of three cases of canine cavitory effusion with confirmed histopathology for splenic and/or hepatic HS were microscopically evaluated.

**Results:** In addition to laboratorial elements consistent with hemorrhage such as erythrocytes to nucleated cells ratio between peripheral blood range, xanthochromic supernatant and macrophages with erythrophagocytosis and/or hemosiderin pigment, rare free spindle cells and a concentration of 5 to 10% of nucleated erythroid cells were observed.

**Conclusions:** The observation of erythroid precursors, such as metarubricytes, in BF samples, or possibly from FNAB of cutaneous nodules, combined with other contextual cytological findings may serve as one of the few specific available resources to indicate HS as a possible differential diagnosis through clinical cytology. Studies involving a larger case pool, including cutaneous lesions, are necessary to verify this hypothesis and to discard the existence of other etiologies that may contribute to the exfoliation of metarubricytes into cavitory effusions.

## **C-21: PATHOPHYSIOLOGY OF GLYCOSURIA IN OBSTRUCTIVE FELINE LOWER URINARY TRACT DISEASE**

Regina Takahira, Roberta Basso, Bruna Santos, Maria Ronchetti

**Background:** Obstructive feline lower urinary tract disease (FLUTD) is one of the major urinary tract emergencies in cats. However, the literature is scarce regarding urinalysis findings in this condition, especially in relation to glucosuria. The hypotheses for this occurrence point to stress hyperglycaemia, diabetes mellitus, tubular lesion and post-renal glucosuria associated with hematuria.

**Objective:** The objective was to elucidate the origin of glucosuria in obstructive FLUTD.

**Methods:** A prospective study was performed with 10 male cats with obstructive FLUTD and glycosuria presented to the Veterinary Hospital at UNESP Botucatu. Serum glucose, fructosamine, glycosylated hemoglobin (HbA1C) and gamma glutamyltransferase (GGT) urinary activity were evaluated. These parameters were compared to a control group (n=9) except for urinary GGT.

**Results:** Although both groups presented hyperglycemia, blood glucose concentration was higher ( $p=0.02$ ) in obstructed cats (mean=162.5 mg/dL) when compared to control group (mean=119.2 mg/dL). Stress hyperglycemia is usually transient in cats with this disease, which may exceed the renal resorption threshold, but none of the animals in this study exceeded this threshold. Diabetes mellitus was also excluded since no animal

presented values of fructosamine above the reference value. Despite the presence of hematuria in all samples, it was not possible to evaluate the contribution of bladder exudation as a source of glycosuria.

**Conclusions:** The low urinary density for dehydrated animals presented by obstructed cats (mean=1.024) suggest a tubular lesion, but the lack of an adequate reference for cat urinary GGT (mean = 32.3 IU/L in obstructed cats) highlights the need of more information

## **C-22: PHENOTYPE REFERENCE DATA OF NORMAL FELINE LYMPH NODES**

Barbara Rütgen, Sabine Hammer, Birgitt Wolfesberger, Sandra Groiss, Daniel Baumgartner, Armin Saalmüller, Ilse Schwendenwein

**Background:** Immunophenotyping by flowcytometric analyses (FCM) is a standard tool for diagnosis and classification of feline lymphoma. However information regarding the physiologic distribution within the lymph node population is scarce.

**Objective:** Aim of the study was to establish preliminary reference data for lymphocyte subpopulations in normal feline lymph nodes. For this purpose, popliteal and/or mandibular lymph nodes from 11 cats euthanized due to various reasons except hematopoietic malignancies were analysed.

**Methods:** Lymph nodes were homogenised into single cell suspensions. Cytospin preparations were stained and evaluated for lymphocyte morphology to ensure their health status. Half of one lymph node was used for histopathological examination. Additionally, polymerase chain reaction for antigen receptor rearrangement of B- and T-cell clonality was performed. The expression of leukocyte differentiation antigens was determined with feline-specific or cross-reactive monoclonal antibodies and analyzed by FCM.

**Results:** Cytology and histopathology revealed a mixed lymphocyte population in all samples and clonality testing confirmed the tumour free status of the material. Lymphocyte populations showed following percentages of positive cells: CD3 iz (55.8%), CD4 (37.5%), CD5 (54.6%), CD8 (12.3%), CD18 (86.9%), CD14 (0.5%), CD21 (38.5%), CD79 $\alpha$ cy (25.9%), CD11d (0.8%).

**Conclusion:** We have established the first reference data for the expression of CD3 iz, CD11d, and CD79 $\alpha$ cy on/in cells derived from feline lymph nodes. Otherwise the results are in accordance with former published data. Differentiation between benign and malignant lymphocyte populations and further insights into different reactive patterns are supported by these results.

## **C-23: PROTEIN BIOMARKERS IN SERUM/URINE FOR EARLY DIAGNOSIS OF CANINE HIP DYSPLASIA**

Chantelle Bozynski, Courtney Moser, Aaron Stoker, Cristi Cook, Emily Leary, James Cook

**Background:** Canine hip dysplasia (CHD) is one of the most commonly diagnosed orthopedic disorders in dogs. According to the Orthopedic Foundation for Animals (OFA) database, the prevalence of CHD can be as high as 70% in certain breeds. Dysplastic dogs are often diagnosed in the irreversible phase (i.e., presence of osteoarthritis), which limits treatment options. Proteins in bodily fluids have been used as biomarkers for early diagnosis of several other disorders, providing potential for disease prevention and improving efficacy of treatment.

**Objective:** To identify protein biomarkers in serum and urine that differentiate dysplastic from normal dogs at an early age (i.e., prior to skeletal maturity).

**Methods:** Whole blood and urine were collected from 14 client-owned dogs at 8 time points (8 weeks to 2 years of age), and 8 biomarkers reflecting direct and indirect measures of joint health were measured. Radiographs were evaluated for joint pathology (hip dysplasia at 2 years: 3 M, 1 F) based on OFA grading criteria. Mann-Whitney U-test and single variable regression analysis were performed with significance set at  $p < 0.05$ .

**Results/Conclusions:** Serum (not urine) biomarker concentrations were significantly different between normal and dysplastic dogs. Male dogs showed a visible distinction in biomarker concentrations between dysplastic and normal dogs for many of the serum biomarkers. Normal females showed a large variability in concentrations, but the dysplastic female mirrored dysplastic male trends. For males, serum biomarkers have the potential to provide early/accurate diagnosis of CHD in puppies. Ongoing analyses will validate this method for clinical use.

#### **C-24: REFERENCE INTERVALS FOR HEMATOLOGICAL AND BIOCHEMICAL VARIABLES AND CYTOLOGIC EVALUATION OF BLOOD AND BONE MARROW IN NSG MALE MICE**

Catherine Layssol-Lamour, Jean-Pierre Braun, Catherine Trumel, Nathalie Bourges-Abella

**Background:** NOD/LtSz-scid IL2R $\gamma$ chain<sup>null</sup> (NSG) mouse is a highly immunodeficient animal model of particular interest in human diseases preclinical studies, for which haematological and biochemical reference intervals (RIs) have not been determined.

**Objective:** The aim of this study was the *de novo* establishment of hematology and biochemical RIs according to ASVCP's recommendations, as well as bone marrow cytology evaluation in NSG male mouse.

**Methods:** According to the principles of 3Rs, the reference sample group was limited to 20 animals. 250  $\mu$ L K3-EDTA and heparin blood specimens as well as blood and femoral bone marrow smears were obtained from 20 nine-week-old NSG males mice. Hematology and biochemistry analyses were performed using ProCytex Dx<sup>®</sup> (Idexx) and VetScan<sup>®</sup> (Scil) analyzers respectively. Blood and bone marrow smears were evaluated for specificities. Distributions of results were tested for normality. RIs (2.5-95.5 limits) and their 90% confidence intervals were determined by robust method.

**Results:** Haematological RIs for the main variables were [7.81-9.20].10<sup>12</sup>/L for Red Blood Cell, [127-150] g/L for Haemoglobin, [51.2-56.6] fL for Mean Corpuscular Volume, [292-312] g/L for Mean Corpuscular Haemoglobin Concentration, [384-546].10<sup>9</sup>/L for Reticulocytes, [0.44-1.43].10<sup>9</sup>/L for Leukocytes. Biochemical RIs for some analytes were [44.0-53.5] g/L for Total Proteins, [30.3-37.4] g/L for Albumin, [3.9-8.4] µmol/L for Bilirubin, [6.2-10.3] mmol/L for Urea, [<18-34.8] µmol/L for Creatinine, [11.3-19.5] mmol/L for Glucose, [43.0-103.0] U/L for ALP and [18.8-36] U/L for ALAT.

**Conclusion:** RIs have been determined according to ASVCP's recommendations on small reference sample groups, thus can be used in cancer research and in interlaboratory comparisons.

## **C-25: SENSITIVITY AND SPECIFICITY OF CYTOLOGIC EVALUATION OF UROTHELIAL CARCINOMA**

Camille McAloney, Samantha Evans, Mary White, Jessica Hokamp, Maxey Wellman

**Background:** Urothelial carcinoma cytology often presents a diagnostic challenge due to inflammation in the urinary tract and rapid degradation of cells in urine. Knowing the diagnostic accuracy of cytology across sampling modalities will allow clinicians to better weigh their risks and benefits. This is particularly relevant given recently-developed, genetics-based diagnostics for this tumor.

**Objective:** Determine the sensitivity and specificity of cytologic evaluation for urothelial carcinoma overall in dogs and cats and among subcategories of sampling method, species, and sex.

**Methods:** Cytology, histopathology, and urinalysis reports in dogs and cats from The Ohio State University Veterinary Medical Center (OSU) and Colorado State University Veterinary Teaching Hospital (CSU) databases were reviewed. Inclusion criteria were cytologic and histologic evaluation of the urinary tract or prostate, performed within three months of each other. Sensitivity and specificity of cytology for the diagnosis of urothelial carcinoma, using histopathology as the gold standard, were calculated for all samples and among subcategories.

**Results:** A total of 326 cases met inclusion criteria. The overall sensitivity and specificity were 93.8% and 50% for OSU and 29.7% and 97.9% for CSU. When only samples reviewed by a pathologist were considered (n=149), less inter-institutional variation was observed. Traumatic catheterization had the highest sensitivity (100%) and specificity (100%) at both institutions.

**Conclusions:** Although traumatic catheterization has a very high diagnostic accuracy, overall cytology is only moderately sensitive and specific for the detection of urothelial carcinoma, underscoring the importance of complementary diagnostic tests. Substantial inter-institutional variation was attributed to differences in pathology review policies.

## **C-26: THE PRESENCE OF LE CELLS IN SYNOVIAL FLUID AS KEY SUPPORT IN THE DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS IN A DOG**

Mara Varvil, Caroline Aldridge, John Christian

Systemic Lupus Erythematosus (SLE) is a multi-organ autoimmune disease whose recognition is challenging, as symptoms are often nonspecific and diagnosis is multifactorial. In dogs, musculoskeletal symptoms are the most frequent primary sign with a symmetric, non-erosive polyarthritis of the distal limbs being most common. Recently, a three-year-old male neutered German Shepherd Dog with a history of arthritis (rDVM) presented to the Purdue University Veterinary Teaching Hospital (PU-VTH) after an episode of weakness with a fever of 106°F, diarrhea, and anemia. The patient's presenting symptoms were treated and an underlying disease process was investigated. Due to the history of a shifting leg lameness, synovial fluid from the right carpus, left carpus, and right tarsus were evaluated for evidence of immune mediate polyarthropathy (IMPA). Cytologic evaluation of these preparations revealed numerous ragocytes, few tart cells, and occasional Lupus Erythematosus (LE) cells. Other evidence for multiorganinvolvement included regenerative anemia, thrombocytopenia and protein-losing nephropathy. Unexpectedly, an echocardiogram revealed evidence of vegetative endocarditis (culture negative), potentially confounding the significance of other results. However, the presence of LE cells in the inflammatory joints was considered strong evidence for SLE and supported subsequent immunosuppressive therapy. Lab abnormalities resolved within one month and the patient is responding well clinically.

#### **C-27: THROMBOELASTOMETRIC CHANGES IN DOGS WITH IMMUNE MEDIATED HEMOLYTIC ANEMIA**

Regina Takahira, Tatiana Gorenstein, Bruna Santos, Pedrita Assunção, Gisele Silva, Grazielly Cunha

**Background:** Immune Mediated Hemolytic Anemia (IMHA) can be secondary to neoplasia, infectious diseases or drugs, whereas a primary IMHA is a diagnosis of exclusion. The hypercoagulable state that predispose to pulmonary thromboembolism is a major cause of death in these animals.

**Objective:** The aim of this work was to evaluate possible changes in the thromboembolic risk of dogs with IMHA, by means of thromboelastometry, at the moment of diagnosis and after treatment.

**Methods:** Seven dogs, two with primary IMHA and five with IMHA secondary to *Ehrlichia canis* were included in the study. Blood samples were obtained in EDTA for CBC, reticulocyte count, saline agglutination test and PCR for *E. canis* and *Babesia canis*; in sodium citrate for thromboelastometry (Rotem®) and serum samples were obtained for leptospirosis serology tests and renal and liver function biochemistry. All animals presenting comorbidities such as neoplasia and chronic renal disease were excluded from the study. All patients were submitted to the same treatment protocol: ranitidine hydrochloride; doxycycline, mycophenolate mofetil and prednisolone. Dogs were evaluated in two moments: M1- moment of diagnosis and M2- after treatment and clinical and hematologic improvement (PCV >30 %).

**Results:** Comparison between two moments was tested by Wilcoxon non parametric test. There was no significant difference ( $p>0.05$ ) among moments M1 e M2 for any of the variables tested, including CT, CFT, alpha angle and MCF.

**Conclusions:** The immunosuppressive treatment did not change the hypercoagulable state presented by dogs with primary or secondary IMHA.

#### **C-28: VIABILITY OF FNA MATERIAL FROM LYMPHOMA CELLS IN DIFFERENT STORAGE MEDIA AND TEMPERATURE**

Barbara Rütgen, Daniel Baumgartner, Armin Saalmüller, Ilse Schwendenwein

**Background:** Immunophenotyping of lymphocyte populations in lymph node material by flow cytometric analysis (FCM) is an indispensable tool for classification of canine lymphoma. Viable cells are required so that analysis should be performed asap at least within 24 hours - this fact limitis the availability of FCM for patients. Commercialized preservative vials developed for man promise viability and marker stability for up to 7 days.

**Objective:** Aim of this study was to compare the usefulness of STRECK cell preservative with other media in canine lymph node material.

**Methods:** Lymph node material submitted for immunophenotyping via FCM was used to test 4 different media:  $1.3 \times 10^6$  cells were transferred into phosphate buffered saline+Gentamycin (PBS/Genta), RPMI1640 cell culture media+Penicillin/Streptomycin (RPMI+P/S), RPMI 1640 cell culture media+10% fetal calf serum+Penicillin/Streptomycin (RPMI+10%FCS+P/S) and 1ml STRECK Cell Preservative™ and stored at 20°C or 4°C respectively. Analysis for viability of the cells was performed on day 0, 1, 3 and 5 using the viability stain Fixable Viability Dye eFluor® 780. Percentage of viable cells was recorded.

**Results:** PBS/Genta followed by RPMI+P/S and RPMI+10%FCS+P/S showed a poorer performance than the STRECK cell Preservative™. Storage temperature seems to be more important regarding viability than the type of media.

**Conclusion:** Cooling is a more stringent requirement for viability than the type of storage media. PBS/Genta showed the worst performance. Data regarding marker stability are still required.

#### **C-29: FIBRINOGEN IN EQUINE PLATELET LYSATE AFFECTS THE CHARACTERISTICS AND FUNCTIONALITY OF MESENCHYMAL STEM CELLS**

Maria Naskou, Scarlett Sumner, Ian Copland, John Peroni

**Background:** Fetal bovine serum (FBS) is used to culture Mesenchymal Stem Cells (MSCs) in the laboratory, however, FBS has been linked to adverse immune-mediated reactions. One alternative to FBS is Platelet Lysate (PL) which promotes MSCs growth without compromising their functionality. Fibrinogen is present in lysate and it can negatively impact MSCs' ability to modulate immune responses.

**Objective:** Our objective was to deplete fibrinogen from equine PL (ePL;fdePL) and compare proliferation, viability and immunomodulatory capacities of MSCs in FBS or ePL with or without fibrinogen.

**Methods:** We manufactured fibrinogen depleted equine platelet lysate (fdePL) from horses and measured specific growth factors via ELISA. LPS-stimulated equine monocytes were cultured with either 10% donor horse serum (DHS), FBS, ePL or fdePL and cell culture supernatants obtained to measure TNF- $\alpha$  via ELISA. We then determined the proliferation, viability and immunomodulatory ability of equine bone marrow-derived MSCs cultured in FBS or ePL with or without fibrinogen. Immunomodulation by MSCs was assessed through TNF-  $\alpha$  production following exposure to stimulated monocytes.

**Results:** Depleting fibrinogen from lysate resulted in a decrease in growth factor concentrations. LPS-stimulated monocytes, in the presence of ePL and fdePL, produced less TNF- $\alpha$  compared to LPS-stimulated monocytes in DHS. MSCs cultured in fdePL exhibited lower proliferation rates but similar viability compared to MSCs in ePL. MSCs grown in ePL reduced TNF- $\alpha$  expression from LPS-stimulated monocytes more effectively than MSCs cultured in fdePL.

**Conclusions:** fdePL suppresses TNF- $\alpha$  expression from LPS-stimulated monocytes but will not support the proliferation and immunomodulatory capacity of MSCs.

## **Experimental Disease Focused Scientific Session I**

November 4, 2018 | 1:30 PM –5:00 PM

Session Chair: Sanjeev Gumber, BVSc, MVSc, PhD, DACVP, Emory University, Atlanta, GA

November 4, 2018

1:30 PM – 1:45 PM

### **IMMUNOPHENOTYPING OF MURINE PRECURSOR B-CELL LEUKEMIA: IMMUNOHISTOCHEMISTRY VERSUS FLOW CYTOMETRY**

Laura Janke, Jerold Rehg

The diagnosis and classification of leukemia in mice often requires the combination of morphologic and immunophenotypic properties. In acute B-cell leukemias, the cells have a generic blastic appearance. Consequently, immunophenotyping is necessary for diagnosis and subtyping. B-cell leukemia can be subtyped based on the expression of markers during normal B-cell development. Flow cytometry is the gold standard by which the marker expression profile for various stages is defined. However, sometimes only formalin-fixed, paraffin-embedded material is available, or flow cytometry results need to be augmented by nuclear or cytoplasmic markers. Therefore it is important to know how well IHC correlates with flow cytometry. A comparison of IHC and flow cytometry has not been undertaken for murine precursor B-cell leukemia. We analyzed 35 cases of chemically- or virally- induced leukemia on which flow cytometry had been performed. Our results indicate that in most cases, IHC generates the same diagnoses as flow cytometry. Most disagreements arose because the transition from Pro B to Pre B is defined by the cytoplasmic expression of IgM (c-mu), which can only be examined by IHC or following membrane permeabilization if done by flow cytometry. Consequently, CD25 membrane expression is commonly used with flow cytometry. Although the expression of CD25 and IgM (c-mu) is correlated in normal B-cell development, our results suggest this is not always the case with leukemia. Based on this comparison, we propose a standard panel of IHC markers to be used to define these subtypes of precursor B-cell leukemia in mice.

November 4, 2018

2:30 PM – 2:45 PM

### **NON-COITAL SEXUAL TRANSMISSION OF ZIKA VIRUS FROM IMMUNOCOMPROMISED MALE MICE IN ACUTE BUT NOT CHRONIC REPRODUCTIVE INFECTION**

Chad Clancy, Arnaud Van Wettere, John Morrey, Justin Julander

**Background:** Zika virus (ZIKV) is a sexually transmitted arboviral infection. The source of infectious virions in the male reproductive tract has yet to be elucidated.

**Objective:** We hypothesize that male reproductive tract epithelial cells are permissive to ZIKV and epididymal fluid or seminal plasma serve as sources of infectious virus for sexual transmission.

**Methods:** Male type I interferon receptor knockout (*Ifnar<sup>-/-</sup>*) mice were challenged subcutaneously with ZIKV. Primary and accessory reproductive tissues were harvested 7, 14, 35 and 70 days post inoculation (DPI) for histopathology. Insemination fluid derived from epididymis or accessory sex glands (seminal plasma) was obtained at 7, 14 and 35 DPI. Type I and II interferon receptor knockout (AG129) females were inoculated intravaginally with epididymal flush or seminal plasma.

**Results:** Testicular pathology initiated as severe orchitis (14 DPI) and progressed to end-stage fibrosing orchitis (35 and 70 DPI). Epididymal inflammation was most severe at 7 DPI, with moderate inflammation at 14 DPI and chronic-active fibrosing epididymitis at 35 and 70 DPI. ZIKV RNA was demonstrated in insemination fluid at 7, 14 and 35 DPI. Viremia of females from each challenge group was observed at 7, but not 14 or 35 DPI. Infectious virus was present in both epididymal fluid and seminal plasma components of insemination fluid at 7, but not 14 and 35 DPI.

**Conclusions:** These studies show passage of virus from epididymal flush and seminal plasma to females via insemination during acute ZIKV disease in males and provides a model for sexual transmission of ZIKV.

November 4, 2018

2:45 PM – 3:00 PM

## **PATHOLOGY OF INTRATRACHEAL AEROSOL INOCULATION WITH BRUCELLA MELITENSIS IN A GUINEA PIG MODEL**

Martha Hensel, Daniel Garcia, Sankar Chaki, Angela Arenas-Gamboa

**Background:** *Brucella melitensis* is a zoonotic, gram negative, intracellular bacterial pathogen that causes splenomegaly, lymphadenomegaly, and adverse pregnancy events in small ruminants with spill-over into people. *B. melitensis* can be transmitted via aerosols and has a low infectious dose in people. A need exists for a biologically relevant model that mimics natural transmission routes and clinical and pathologic features of disease.

**Objective:** To develop the guinea pig as an aerosol model of infection for brucellosis.

**Methods:** The PennCentury MicroSprayer Aerosolizer was used to inoculate female guinea pigs with 16M *B. melitensis* at  $1 \times 10^3$ /50 ml (low dose, n=4) or  $1 \times 10^7$ /50 ml (high dose, n=4). At 30 days post-infection, spleen, liver, lung, cervical lymph node, tracheobronchial lymph node, and uterus were evaluated for gross and histopathologic lesions. Histologic scores were assigned, and immunohistochemistry of the spleen and uterus was performed.

**Results:** Intratracheal inoculation resulted in systemic infection characterized grossly by splenomegaly, lymphadenomegaly, and hepatic necrosis. Histologically, inflammation in all tissues was predominantly histiocytic with fewer neutrophils. The liver also had random foci of necrosis with a lymphoplasmacytic periportal infiltrate. The high dose group had a higher average histologic score compared to the low dose group. *Brucella* antigen was detected in the spleen and uterus by IHC.

**Conclusions:** The guinea pig can be infected via intratracheal aerosol inoculation and develops systemic lesions of brucellosis that mimic natural disease. This the first report of *Brucella* spp. tropism for the non-gravid uterus and suggests that guinea pigs could be models for *Brucella*-associated reproductive disease.

November 4, 2018

4:30 PM – 4:45 PM

#### **CHARACTERIZATION OF BLOOD-TUMOR BARRIER PATHOLOGY IN A MOUSE MODEL OF BRAIN METASTASES OF LUNG CANCER**

Alexandra Dieterly, Gozde Uzunalli, Chinyere Kemet, Arvin Soepriatna, Craig Goergen, Michael Wendt, Tiffany Lyle

The incidence of brain metastases of lung cancer is increasing. Current treatment of brain metastases is ineffective due to poor uptake and the transformation of the blood-brain barrier (BBB) into the blood-tumor barrier (BTB). We identified altered functional components of the BBB in a mouse model non-small cell lung cancer (NSCLC) brain metastases. A brain-seeking variant of NSCLC was injected into the left cardiac ventricle of nu/nu mice and brains were harvested within six-weeks. In each mouse the BBB and BTB were qualitatively and quantitatively analyzed using immunofluorescence microscopy. We report a 5.4-fold decrease in CD31 (endothelial cell) expression in the BTB compared to the BBB. A 1.7-fold decrease in the tight junction adapter protein, zona occludens-1 was present, in the BTB compared to the BBB. The parenchymal basement membrane was altered in the BTB, exhibiting a 7.4 fold decrease in laminin- $\alpha$ 2 expression. A loss of multiple pericyte subtypes was identified in the BTB. We identified a 2.6-fold decrease in expression of PDGFR- $\beta$ , a pan-pericyte protein, within the BTB. There was a 7.5-fold decrease in resting pericytes and a 2.0-fold decrease in contractile pericytes. Similar pathologic changes were identified in the BTB at five weeks post-intracardiac injection. Our findings suggest that the tight junction adaptor protein, parenchymal basement membrane, and pericytes are critical in maintaining physiologic integrity of the BBB. A distinct set of BBB alterations occur in the brain metastases of lung cancer, and tight junctions, basement membranes, and pericytes may be effective chemotherapeutic targets to improve patient survival.

November 4, 2018

4:45 PM – 5:00 PM

#### **SNAKE FUNGAL DISEASE: A NOVEL EXPERIMENTAL MODEL**

Christina McKenzie, Paul Oesterle, Claire Jardine, Christina Davy, Brandon Lillie, Nicole Nemeth

**Background:** Snake fungal disease (SFD) is an important disease in captive and free-ranging snakes worldwide. SFD was recognized as a distinct entity in 2011 as a cause of dermatitis and death in wild snakes. *Ophidiomyces ophiodiicola* has been confirmed as the cause of SFD through experimental studies. Developing a reliable and relevant experimental model is integral to furthering our understanding of SFD. Previous experimental models have used inoculation of nasolabial pits and scarification as means of exposure but resulted in unknown infectious dose and rapid clearing of infection after skin shedding.

**Objectives:** We sought to replicate SFD in corn snakes via subcutaneous injection.

**Methods:** Juvenile corn snakes (24 exposed, 7 control) were subcutaneously injected with *O. ophiodiicola* conidia and monitored for lesion development and fungal detection on skin. Subsequently, snakes underwent simulated brumation followed by euthanasia at 10 weeks post-inoculation (PI). Select tissues and serially collected skin swabs were tested by PCR and examined microscopically.

**Results:** Infected snakes developed gross (10/24) and microscopic (21/24) skin lesions consistent with SFD at inoculation sites. Two snakes had microscopic skin lesions distant to inoculation sites. Most snakes developed systemic fungal infection (21/24), including granulomas in liver, coelomic connective tissue, thymus and lung. Tissues from most (18/18) snakes tested PCR-positive and fungus was detected on skin swabs of 10/24 snakes at varied time points PI (up to 10 weeks PI). 3/8 shed skins tested PCR-positive.

**Conclusion:** Subcutaneous inoculation of *O. ophiodiicola* in corn snakes created lesions of SFD and often caused systemic fungal infection.

## Experimental Disease Focused Scientific Session II

November 6, 2018 | 1:30 PM –5:00 PM

Session Chair: Sanjeev Gumber, BVSc, MVSc, PhD, DACVP, Emory University, Atlanta, GA

November 6, 2018

1:30 PM – 1:45 PM

### **MURINE MODELS OF LUPUS NEPHRITIS AND EFFICACY OF SERPINB3 FOR IMMUNOMODULATORY RESPONSE**

Giuseppe Maggioni, Anna Ghirardello, Roberto Luisetto, Andrea Doria, Laura Cavicchioli

**Background:** Lupus-prone mouse strains, New Zealand Black/White (NZB/NZW F1) and Murphy Roth Large//*pr* are widely used animal models in SLE pathophysiology, clinical and drug development study, especially for lupus nephritis (LN). SERPINB3 is tested for therapeutic purposes and effective dose in LN immunomodulatory response.

**Methods:** 16 NZB/NZW F1 and 12 MRL//*pr* mice were subdivided in groups and intraperitoneally injected with human recombinant SERPINB3 in a prophylactic (before proteinuria traces onset administration of SERPINB3 7.5 µg/0.1mL) or therapeutic approach (after frank proteinuria onset -300 mg/dl-, administration of SERPINB3 15µg/0.1mL). Control mice were administered with PBS (0.1 mL). Harvested kidneys were collected and FFPE slides were examined with histological (HE, PAS, PASM, Masson's Trichrome) and IHC (anti-CD3, anti-CD20, anti PD-1, antiPDL-1) stains. Kidney lesions were scored and statistically analyzed in the light of clinical data.

**Results:** The histopathologic score system applied to LN in different lupus-prone murine strains is solid. The clinical finding more strongly correlated with score system items and indexes is murine proteinuria. The infiltrating lymphocytes, mainly deposited around arcuate artery, are T-cells CD3<sup>+</sup>, PD1<sup>+</sup> and scarcely-PDL1<sup>+</sup>, suggesting an active proliferation and damage to nephron.

NZB/NZW F1 strain proved to be a reliable model to study renal involvement in SLE and especially LN. MRL//*pr* mice on the other hand, show a quicker clinical and histopathological disease onset.

**Conclusions:** With a statistic relevant significance, SERPINB3 administration plays a pivotal role in delaying death in treated mice, and shows a positive trend, worth further investigations, in delaying, at least, histopathological damage.

November 6, 2018

2:30 PM – 2:45 PM

### **ROLES OF ANTIBODY IN SUPPRESSION OF PANCREATIC CANCER PROGRESSION AND METASTASIS**

Jeremy Foote, Christopher Klug

**Background:** Recent evidence indicate significant roles for B cells in promoting pancreatic cancer through mechanisms involving B-cell activating factor, interleukin-35, and antibody-antigen immune complexes, which supports pancreatic ductal adenocarcinoma (PDAC) cell survival, proliferation, and generation of immune suppressive M2-macrophage within the pancreas. Significant deficits in our knowledge of anti-tumor effects of B cells in pancreatic cancer remain, in particular, the involvement of tumor-specific antibodies in all stages of pancreatic cancer progression.

**Objective:** Evaluate impact of global antibody deficiency on spontaneous pancreatic cancer progression using mice with pancreatic-specific mutations in Kras alone (KC) or in addition to p53 heterozygosity (KPC).

**Methods:** Survival in WT and antibody deficient KPC mice were monitored over the course of 10 months and both PDAC formation and distal metastasis were evaluated on hematoxylin and eosin stained sections of pancreas, lung, liver at 3 and 4 months of age and at time of euthanasia due to progressive disease. Immune cell subsets present in the pancreas of control, WT and antibody deficient KPC and KC mice were evaluated at 3 and 5 months, respectively.

**Results:** Antibody deficiency accelerates pancreatic intraepithelial neoplasm and PDAC formation, decreasing overall survival, and increasing rate of metastasis to lungs and liver. Antibody deficient KC and KPC mice display increased numbers of intrapancreatic CD1d<sup>hi</sup> CD5<sup>+</sup> B cells, Foxp3<sup>+</sup> regulatory T cells, and CD206<sup>+</sup> M2-polarized macrophage while exhibiting decreasing proportions CD8<sup>+</sup> cytotoxic T cells.

**Conclusions:** Antibody limits spontaneous PDAC formation and metastasis in KPC mice potentially through modulating tumor-specific immune responses beginning within pre-malignant stages.

November 6, 2018  
2:45 PM – 3:00 PM

## **CHARACTERIZATION OF SPONTANEOUS LYMPHOMAS/LEUKEMIAS IN NSG MICE**

Heather Tillman, Laura Janke, Peter Vogel, Jerold Rehg

The NOD.Cg-*Prkdc*<sup>scid</sup> *Il2rg*<sup>tm1Wj</sup>/SzJ strain (i.e., NOD *scid* gamma, NSG) is a severely immunodeficient, laboratory mouse used for preclinical studies because it is amenable to engraftment of human cells. Combined *Scid* and *IL2rg*<sup>null</sup> mutations impair maturation and functionality of T, B and NK lymphocytes. NSG mice are more resistant to developing spontaneous lymphomas/leukemias because “leakiness” is reduced when the functions of the common cytokine receptor gamma-chain subunit are lost. There is one report of spontaneous lymphomas/leukemias occurring in 3 NSG mice (~2.4% incidence) over a period of 104 weeks. Two of these lymphomas were classified as a B cell lineage, with one being a T cell lineage. In this study, we characterized the immunophenotypes of 10 spontaneous lymphomas/leukemias diagnosed in NSG mice (~3.4% incidence) having combinations of an enlarged thymus, splenomegaly and lymphadenopathy and variable histologic involvement of the bone marrow, lung, and

other tissues. Neoplastic cells were CD3 positive with mixtures of CD4 and CD8 positive cells, which was consistent with a precursor T cell lymphoblastic lymphoma/leukemia phenotype (pre-T LBL). Both males (2) and females (8) were affected with ages ranging from 14 to 24 weeks. T cell lymphomas/leukemias developed both in unmanipulated breeders housed under SPF conditions and in mice used for engraftment studies. These data highlight the permissive nature of T lymphocyte development by showing that bone marrow T cell precursors may undergo pre-TCR mediated expansion and neoplastic transformation especially when there is residual DNA-dependent protein kinase function contributed by the *Scid* phenotype.

November 6, 2018

4:30 PM – 4:45 PM

### **ZIKA VIRUS AND TESTICULAR TERATOMAS IN MICE: INSIGHTS INTO VIRUS TROPISM AND DISEASE PRESENTATION IN HUMANS**

Ian Moore

Zika viruses are members of the family *Flaviviridae* and are spread by mosquitoes. Zika virus has been circulating in the forests of Uganda, where it was first discovered, since 1947. Recently, this virus has spread towards the Americas, resulting in a Zika virus epidemic which has largely affected South America, the Caribbean and the Southern United States. The pandemic resulted in severe disease that was most prominent in pregnant women, with mild affects to the mother but devastating neurologic developmental issues in the neonate, in some cases resulting in neonatal death. Interestingly, it has been reported that infection in women can occur through mosquito transmission or via sexual transmission from a partner. Based on these modes of transmission, researchers investigated the virus' tropism for reproductive-associated tissues and have performed several animal studies to further elucidate the course of infection. In mice, the virus can replicate in the testes and is associated with inflammation and tissue damage. Moreover, in mouse strains with a high incidence of testicular teratomas, we observed that, in addition to infection of resident testicular tissue, cell types consistent with CNS, cartilage and bone present in the tumor, were virus antigen positive when evaluated by immunohistochemistry. Additionally, *in situ* hybridization (ISH) confirmed Zika virus RNA in the same cell populations. These findings taken with the severe neurological manifestations, common to children after intra-uterine Zika virus infection, for which little is known about the pathogenesis, may highlight notable features of virus tropism that are relevant in human disease.

November 6, 2018

4:45 PM – 5:00 PM

### **CONSERVED IMPACT OF BRCA2 MUTATION ON PLOIDY OUTCOME IN ZEBRAFISH CANCERS**

Leroy Mensah, Jordan Ferguson, Heather Shive

**Background:** The tumor suppressor *BRCA2* is essential for maintaining genomic integrity, and mutations in *BRCA2* result in chromosomal instability (CIN) and increased cancer risk. Although a major outcome of CIN is aneuploidy, *BRCA2*-associated human

cancers are often diploid. However, unlike many human cancer types, diploidy is a negative prognostic factor in *BRCA2*-associated cancers.

**Objective:** We used a *brca2*-mutant zebrafish model to further investigate the impact of *BRCA2* mutation on ploidy and survival outcome in vertebrate cancer.

**Methods:** We analyzed cancers from 99 *brca2*-mutant/*tp53*-mutant and *tp53*-mutant zebrafish siblings to compare ploidy outcomes in *brca2*-associated (*brca2*-mutant/*tp53*-mutant) and non-*brca2*-associated (*tp53*-mutant) cancers. DNA content analysis was performed on matched normal and cancer specimens from each zebrafish, and ploidy was determined by calculating DNA index.

**Results:** In zebrafish cancers, ploidy outcome was influenced by both *brca2* genotype and sex. Diploidy was more common in *brca2*-associated cancers than in non-*brca2*-associated cancers. Aneuploidy was more common in females than males, particularly in non-*brca2*-associated cancers. Survival time was significantly lower in zebrafish with *brca2*-associated cancers and in females from both genotypic groups. Analysis of combined factors (*brca2* genotype, ploidy outcome, sex) demonstrated that the lowest median survival times occurred in zebrafish with (1) *brca2* mutation and diploid cancers and (2) *brca2* mutation and female sex.

**Conclusions:** These findings support a specific link between diploidy and poor survival outcome in the context of *BRCA2* mutation, and suggest sex as contributor to prognosis in individuals with *BRCA2*-associated cancers.

## Experimental Disease Focused Scientific Poster Session

### E-01: THE EFFECT OF PORCINE EPIDEMIC DIARRHEA VIRUS INFECTION ON TIGHT JUNCTION EXPRESSION IN THE SMALL INTESTINE OF WEANED PIGS

Ya-Mei Chen, Nicholas Gabler, Jesse Hostetter, Eric Burrough

**Background:** Porcine epidemic diarrhea virus (PEDV) infection in swine causes loss of enterocytes, villus blunting, diarrhea, and digestive dysfunction. Previous studies have demonstrated PEDV infection impairs intestinal barrier integrity in nursery pigs.

**Objective:** The aim of this study is to evaluate restitution of the small intestine in weaned pigs after PEDV infection.

**Methods:** Sixty-four 8-week-old pigs were randomly arranged in two treatments [PEDV-infected (n = 40) and mock (n = 24)] and orally inoculated with PEDV IN19338 strain or mock. At day after inoculation (dpi) 2, 4, 6, and 10, pigs were euthanized, and jejunum, ileum, tonsil, spleen, and mesenteric lymph node were collected for histological examination and detection of PEDV and Zonula occluden (ZO)-1 by immunohistochemistry.

**Results:** Results show that PEDV antigen was detected in PEDV-inoculated pigs in 30% (3/10) at dpi 4, 40% (4/10) at dpi 6, and 20% (2/10) at dpi 10. Compared with the mock, only PEDV-infected pigs at dpi 4 and 6 showed shortened ( $p < 0.05$ ) villi in the jejunum. However, using Halo image analysis, PEDV-inoculated pigs at dpi 10 revealed lower ( $p < 0.05$ ) expression of ZO-1 in both jejunum and ileum.

**Conclusions:** ZO-1 is one of the tight junction proteins expressed between enterocytes and is crucial for maintaining intestinal integrity. These data suggest, in weaned pigs, PEDV infection persistently reduces tight junction expression, even after apparent villus recovery, and may thereby continue to reduce barrier function to at least dpi 10.

### E-02: HISTOLOGIC CHARACTERIZATION OF MALE REPRODUCTIVE PATHOLOGY DURING ACUTE ZIKA VIRUS INFECTION IN IMMUNOCOMPROMISED MICE

Chad Clancy, Arnaud Van Wettere, Venkatraman Siddharthan, John Morrey, Justin Julander

**Background:** Phenotypic characterization of the male reproductive tract histopathology may allow further understanding of the pathogenesis of infection with Zika virus and sexual transmission.

**Objective:** Our objective was to characterize the disease progression, identify cellular targets and identify potential routes of sexual transmission of ZIKV infected males.

**Methods:** Two common immunocompromised mouse strains used in transmission studies: one with interferon types I and II receptor genes knocked out (AG129) and one with interferon type 1 receptor knocked out (*Ifnar<sup>-/-</sup>*) were infected with a contemporary

Puerto Rican Zika virus isolate (PRVABC59). Inflammation severity was assessed 5 to 11 days after infection using a histopathology grading system.

**Results:** ZIKV antigen and RNA was initially detected in epididymal epithelial cells in both male models. ZIKV antigen was demonstrated by immunohistochemistry in the prostate and seminal vesicle of infected AG129 mice. Inflammation was observed in both models; however, histopathology was more severe in AG129 males. Inflammation initiated as epididymitis and progressed to orchitis in both AG129 and *Ifnar*<sup>-/-</sup> males.

**Conclusion:** Time-course analysis of infection revealed an increase in the severity of disease and an increased rate of detection within the lumen of the epididymis of both strains, indicating a potential route of sexual transmission. Infection of *Ifnar*<sup>-/-</sup> mice may better recapitulate Zika virus pathology in humans due to milder histopathologic lesions, the presence of histologically normal sperm in epididymal tubules, and an ability to survive the acute phase of disease. This work provides important information for sexual transmission studies in an animal model.

### **E-03: APOLIPOPROTEIN A-I DETECTION IN CSF OF RABBIT EXPERIMENTALLY INOCULATED WITH RABIES VIRUS AND ITS EXPRESSION IN THE BRAIN**

Yuji Sunden, Midori Kihara, Naoki Tsunekawa, Takehito Morita

**Background:** Rabies is a classical zoonotic disease, however, still have undefined pathogenesis. The detailed proteomics of cerebrospinal fluid (CSF) after rabies virus (RV) exposure are not investigated.

**Objective:** To detect protein expression in CSF collected from rabbits experimentally inoculated RV. The protein expression in rabid rabbit's brains was examined to understand the roles between CSF changes and rabies pathogenesis.

**Methods:** NZW rabbits were inoculated with 10<sup>6</sup> PFU of RV (CVS) into the muscles of hind legs. CSF collection was performed before inoculation (control), on 4 and 9 days post inoculation (dpi). The band of protein, which was not observed in control and 4dpi, was extracted to analyze the amino acid sequences. Histopathological analysis and immunohistochemistry of brains were also performed.

**Results:** The rabbits showed progressive paralysis from 5-6 dpi, and were dead or endpoint on 10-12 dpi. Peptide sequence of apolipoprotein A-I (Apo A-I) was identified in CSF of 9 dpi rabbits. On histopathological section, diffuse severe non-suppurative encephalitis was observed and positive signals of Apo A-I were found mainly in glial cells those were expressed Iba-1, but not GFAP.

**Conclusions:** Apo A-I was found in CSF and was expressed in microglia of rabid rabbits. Apo A-I is one of a factor of high-density lipoprotein, and the significance of Apo A-I in rabies is still unclear. However, variable expression of Apo A-I in brain of patients suffering from neurodegenerative disorders and repair promotion in CNS damage has been reported.

#### **E-04: SNAKE FUNGAL DISEASE: A NOVEL EXPERIMENTAL MODEL**

Christina McKenzie, Paul Oesterle, Claire Jardine, Christina Davy, Brandon Lillie, Nicole Nemeth

**Background:** Snake fungal disease (SFD) is an important disease in captive and free-ranging snakes worldwide. SFD was recognized as a distinct entity in 2011 as a cause of dermatitis and death in wild snakes. *Ophidiomyces ophiodiicola* has been confirmed as the cause of SFD through experimental studies. Developing a reliable and relevant experimental model is integral to furthering our understanding of SFD. Previous experimental models have used inoculation of nasolabial pits and scarification as means of exposure but resulted in unknown infectious dose and rapid clearing of infection after skin shedding.

**Objectives:** We sought to replicate SFD in corn snakes via subcutaneous injection.

**Methods:** Juvenile corn snakes (24 exposed, 7 control) were subcutaneously injected with *O. ophiodiicola* conidia and monitored for lesion development and fungal detection on skin. Subsequently, snakes underwent simulated brumation followed by euthanasia at 10 weeks post-inoculation (PI). Select tissues and serially collected skin swabs were tested by PCR and examined microscopically.

**Results:** Infected snakes developed gross (10/24) and microscopic (21/24) skin lesions consistent with SFD at inoculation sites. Two snakes had microscopic skin lesions distant to inoculation sites. Most snakes developed systemic fungal infection (21/24), including granulomas in liver, coelomic connective tissue, thymus and lung. Tissues from most (18/18) snakes tested PCR-positive and fungus was detected on skin swabs of 10/24 snakes at varied time points PI (up to 10 weeks PI). 3/8 shed skins tested PCR-positive.

**Conclusion:** Subcutaneous inoculation of *O. ophiodiicola* in corn snakes created lesions of SFD and often caused systemic fungal infection.

#### **E-05: NON-COITAL SEXUAL TRANSMISSION OF ZIKA VIRUS FROM IMMUNOCOMPROMISED MALE MICE IN ACUTE BUT NOT CHRONIC REPRODUCTIVE INFECTION**

Chad Clancy, Arnaud Van Wettere, John Morrey, Justin Julander

**Background:** Zika virus (ZIKV) is a sexually transmitted arboviral infection. The source of infectious virions in the male reproductive tract has yet to be elucidated.

**Objective:** We hypothesize that male reproductive tract epithelial cells are permissive to ZIKV and epididymal fluid or seminal plasma serve as sources of infectious virus for sexual transmission.

**Methods:** Male type I interferon receptor knockout (*Ifnar<sup>-/-</sup>*) mice were challenged subcutaneously with ZIKV. Primary and accessory reproductive tissues were harvested 7, 14, 35 and 70 days post inoculation (DPI) for histopathology. Insemination fluid

derived from epididymis or accessory sex glands (seminal plasma) was obtained at 7, 14 and 35 DPI. Type I and II interferon receptor knockout (AG129) females were inoculated intravaginally with epididymal flush or seminal plasma.

**Results:** Testicular pathology initiated as severe orchitis (14 DPI) and progressed to end-stage fibrosing orchitis (35 and 70 DPI). Epididymal inflammation was most severe at 7 DPI, with moderate inflammation at 14 DPI and chronic-active fibrosing epididymitis at 35 and 70 DPI. ZIKV RNA was demonstrated in insemination fluid at 7, 14 and 35 DPI. Viremia of females from each challenge group was observed at 7, but not 14 or 35 DPI. Infectious virus was present in both epididymal fluid and seminal plasma components of insemination fluid at 7, but not 14 and 35 DPI.

**Conclusions:** These studies show passage of virus from epididymal flush and seminal plasma to females via insemination during acute ZIKV disease in males and provides a model for sexual transmission of ZIKV.

#### **E-06: PATHOLOGY OF INTRATRACHEAL AEROSOL INOCULATION WITH BRUCELLA MELITENSIS IN A GUINEA PIG MODEL**

Martha Hensel, Daniel Garcia, Sankar Chaki, Angela Arenas-Gamboa

**Background:** *Brucella melitensis* is a zoonotic, gram negative, intracellular bacterial pathogen that causes splenomegaly, lymphadenomegaly, and adverse pregnancy events in small ruminants with spill-over into people. *B. melitensis* can be transmitted via aerosols and has a low infectious dose in people. A need exists for a biologically relevant model that mimics natural transmission routes and clinical and pathologic features of disease.

**Objective:** To develop the guinea pig as an aerosol model of infection for brucellosis.

**Methods:** The PennCentury MicroSprayer Aerosolizer was used to inoculate female guinea pigs with 16M *B. melitensis* at  $1 \times 10^3$ /50 ml (low dose, n=4) or  $1 \times 10^7$ /50 ml (high dose, n=4). At 30 days post-infection, spleen, liver, lung, cervical lymph node, tracheobronchial lymph node, and uterus were evaluated for gross and histopathologic lesions. Histologic scores were assigned, and immunohistochemistry of the spleen and uterus was performed.

**Results:** Intratracheal inoculation resulted in systemic infection characterized grossly by splenomegaly, lymphadenomegaly, and hepatic necrosis. Histologically, inflammation in all tissues was predominantly histiocytic with fewer neutrophils. The liver also had random foci of necrosis with a lymphoplasmacytic periportal infiltrate. The high dose group had a higher average histologic score compared to the low dose group. *Brucella* antigen was detected in the spleen and uterus by IHC.

**Conclusions:** The guinea pig can be infected via intratracheal aerosol inoculation and develops systemic lesions of brucellosis that mimic natural disease. This the first report of *Brucella* spp. tropism for the non-gravid uterus and suggests that guinea pigs could be models for *Brucella*-associated reproductive disease.

## **E-07: INTESTINAL T CELL RESPONSES DURING EARLY EXPERIMENTAL MAP INFECTION OF CALVES**

Rebecca Egan, Kevin Stinson, Latasha Ludwig, Brandon Plattner

*Mycobacterium avium* subspecies *paratuberculosis* (Map) is an obligate intercellular bacterium that causes Johne's disease, a progressive and invariably fatal disease of ruminants. Map persists in intestinal macrophages during a protracted subclinical phase, and infected animals eventually progress to clinical disease characterized by malabsorptive diarrhea, wasting, and death. Evidence suggests that a small percentage of Map-exposed calves progress to clinical disease, and it is hypothesized that some animals are resilient to infection; however, little is known about early cellular immune responses, host-pathogen interactions, and mechanisms of the anti-Map response at the site of infection. Data have shown that T cell subsets, including gamma delta ( $\gamma\delta$ )<sup>+</sup> and CD8<sup>+</sup> cells, are recruited during Map infection, but their function remains unclear. Initial host responses at sites of Map infection are hypothesized to play a significant role in resilience to Map infection, and the aim of this study was to characterize early cellular immune responses in the calf intestine following experimental intestinal inoculation. Fluorescence microscopy was utilized to assess expression of CD3,  $\gamma\delta$ , WC1, and CD8a on cells within the ileal mucosa during the first seven months following inoculation, and gene expression of IFN- $\gamma$  and IL-10 within ileal tissue was measured by digital droplet PCR. These were correlated with histopathologic lesions and persistence of Map in tissue, which demonstrated differences between groups based on Map status. This study provides significant insight into the early local host immune response following enteric Map infection, and factors that may contribute to resilience or persistence of Map infection.

## **E-08: INFECTION OF NOD.SCID.IL2RG-/- MICE WITH NON-MOUSE-ADAPTED SWINE-ORIGIN AND HUMAN-ORIGIN H1 AND H3 INFLUENZA A VIRUSES**

Joshua Lorbach, Sarah Nelson, Sarah Lauterbach, Jacqueline Nolting, Andrew Bowman

**Background:** Influenza A viruses (IAV) emerging from animal reservoirs pose pandemic influenza threat. Humanized mice are used in IAV vaccine development, though use of this model to study existing human anti-IAV immunity against emerging IAVs could improve pandemic risk assessment. It is widely accepted that IAV infection in immunocompetent mice requires serial passage, though susceptibility of immunodeficient mice is unclear. Further, mouse-adapted IAV may not accurately predict wild-type disease.

**Objective:** Our objective was to investigate susceptibility of an immunodeficient mouse strain to infection with non-adapted human- and swine-origin H1 and H3 IAVs.

**Methods:** Pairs of adult, female NOD.SCID.*Il2rg*<sup>-/-</sup> (NSG) mice were intranasally inoculated with one of six IAVs [swine-origin (H1 delta1, H1 gamma, H3 human-like, H3 cluster IV), human seasonal (H1N1, H3N2)] ( $10^5$ - $10^6$  TCID<sub>50</sub>) or PBS and monitored for weight loss and clinical signs for 96 hours post-inoculation (hpi). Postmortem lung tissue samples were analyzed by histology and qRT-PCR (matrix).

**Results:** qRT-PCR detected IAV in lung tissue from all virus-inoculated mice. Clinical signs ranged from none to rough fur coat and hunched posture. Weight loss ranged from 2.4% to >15%, with some mice requiring early removal (72 hpi). Microscopic lesions included acute bronchial epithelial necrosis and leukocyte infiltration.

**Conclusions:** These findings support the general potential of non-mouse-adapted IAV isolates to cause clinical and microscopic disease in NSG mice, contradicting the general consensus that IAV is not naturally infectious in mice. Furthermore, these data support continued exploration of the humanized mouse as a model of natural human anti-IAV immunity.

#### **E-09: TROPISM OF BRUCELLA SPP. TO THE FEMALE REPRODUCTIVE TISSUES IS NOT ASSOCIATED WITH PREGNANCY**

Shakirat Adetunji, Omar Khalaf, Martha Hensel, Angela Arenas-Gamboa

**Background:** Tropism of *Brucella* spp. to the pregnant female reproductive system and the associated adverse effects of spontaneous abortion and stillbirth, especially in livestock, have been established for more than a century. However, little is known about the detrimental effect of *Brucella* infection on the non-pregnant uterus.

**Objective:** The objective of this current study was to determine if *Brucella* could infect non-pregnant uterus, sustain infection, and cause any type of inflammatory changes.

**Methods:** 8-10-week-old ICR mice (n=30) were inoculated intraperitoneally with live attenuated vaccine strain *B. abortus* S19 and the wildtype *B. abortus* 2308 at a dose of  $10^6$  CFU/mouse. Uterine tissue samples collected at 3, 7, and 13 days post infection (dpi) were analyzed for routine bacterial isolation, histopathology, immunohistochemistry, and fluorescent *in situ* tissue hybridization.

**Results:** Colonization and tropism to the uterus of non-pregnant mice occurred as early as 3dpi and was confirmed by the recovery of *Brucella*, as well as via immunohistochemical and fluorescent *in situ* hybridization labelling of *Brucella* antigen in all infected mice. Histologically, mild to moderate neutrophilic and histiocytic endometritis was evident at 7dpi and it progressed.

**Conclusions:** This is the first report to confirm that the tropism of *Brucella* to the female reproductive system is independent of pregnancy. Further investigation is required to determine the mechanisms of immune cell trafficking to the uterus during *Brucella* infection and how that impacts implantation success.

#### **E-10: CALIFORNIA SEA LION (ZALOPHUS CALIFORNIANUS) LYMPH NODE EXPLANT REVEALS INVOLVEMENT AND TRANSCRIPTIONAL REGULATION OF THE CELL ENTRY RECEPTORS SLAM AND NECTIN-4 DURING PHOCINE DISTEMPER VIRUS INFECTION**

Omar Gonzales-Viera, Tracey Goldstein, Piyaporn Eiamcharoen, Pdraig Duignan, Michael Keel

**Background:** Phocine distemper virus (PDV) is the culprit of thousands of pinniped deaths. Even though, the PDV-associated mortalities have been extensively investigated, the pathogenesis at the cellular level is scarce.

**Objective:** To investigate whether PDV uses the cell entry receptors SLAM or nectin-4 to infect the California sea lion (CSL) lymph node and whether PDV induces the transcriptional regulation on both receptors during the lymph node infection. We investigated the PDV-induced apoptosis.

**Methods:** We used a lymph node explant culture system from CSL. Lymph nodes were collected, infected by PDV and maintained *in vitro* for 5 days.

**Results:** PDV infected and replicated in the lymph nodes with a replication peak on the third day post infection (dpi). The PDV+ cells colocalized SLAM and nectin-4 in their cytoplasm and those cells were IBA1+ (histiocytic lineage). Comparison of receptor expression between infected and mock-infected lymph nodes revealed the transcriptional downregulation of both receptors during the first 2 dpi and upregulation the last 2 dpi. We observed higher number of apoptotic cells (caspase-3+ cells) in the infected lymph nodes and they coexpressed PDV in the cytoplasm.

**Conclusion:** The lymph node explant is an important model to study the PDV pathogenesis in lymph nodes. The explant demonstrated that PDV induces the transcriptional regulation of SLAM and nectin-4 in lymph nodes as a strategy to reduce over infection of the host cell and to evade the innate immune system. We presented evidences that PDV induces apoptosis in the lymph node as a form of immunosuppression.

## **E-11: CYTOKINE SIGNALING THROUGH DROSOPHILA MTHL10 TIES LIFESPAN TO ENVIRONMENTAL STRESS**

Eui Jae Sung, Yoichi Hayakawa, Stephen Shears

Invertebrates are productive models for understanding basic molecular principles that link cytokine-mediated, inflammatory pathways to aging in humans. Here, we employed *Drosophila*, a routine model for analysis of cytokine signaling pathways in higher animals, to identify a receptor for the growth-blocking peptide (GBP) cytokine, known as a critical regulator of humoral and cellular innate immune responses and metabolic homeostasis. We established that the phospholipase C/ $\text{Ca}^{2+}$  signaling pathway mediates innate immune responses to GBP, and have conducted a high-throughput dsRNA library screen to determine genes that modulate  $\text{Ca}^{2+}$  mobilization in *Drosophila* S3 cells. We found that a hitherto orphan G protein coupled receptor, Methuselah-like receptor-10 (Mthl10), was a significant hit. A series of secondary screening experiments confirmed the role of Mthl10 in  $\text{Ca}^{2+}$  mobilization and specific binding of fluorophore-tagged GBP to Mthl10 in S3 cell. Fluorescence polarization was used to demonstrate direct binding of tagged-GBP to recombinant Mthl10-ectodomain. In adult flies, Mthl10 knockdown decreases GBP-mediated innate immune responses and increases mortality rate following infection with *Micrococcus luteus* in adult flies. On the other hand, elevated GBP expression - a mimetic for a stressful environment - also led to a

lifespan reduction. However, in the absence of pathogenic challenges, Mthl10 knockdown significantly extends lifespan. Our results provide a molecular basis for hypothesizing that a successful defense against inflammation can be a Pyrrhic victory that reduces longevity. Finally, similarities between GBP and human defensin BD2, in both primary sequence and molecular actions, indicate our stress/longevity model may be applicable to humans.

#### **E-12: ENHANCING THE IMMUNOGENICITY OF A RECOMBINANT LACTOBACILLUS ACIDOPHILUS ORAL VACCINE AGAINST HIV-1 THROUGH THE EXPRESSION OF THE E. COLI TYPE I PILUS PROTEIN FIMH**

Allison Vilander, Akinobu Kajikawa, Gregg Dean

**Background:** *Lactobacillus acidophilus* (LA) is a Gram positive lactic acid bacterium used in food and probiotic products. We have developed an oral recombinant LA (rLA) vaccine platform with surface expression of a weak immunogen, the HIV-1 membrane proximal external region (MPER). To increase the immunogenicity, we surface expressed the *E. coli* type I pilus protein FimH, a Toll-like receptor 4 ligand and microfold cell targeting protein that can enhance uptake into Peyer's patches (PP).

**Objective:** Evaluate the immune response following rLA-MPER+FimH oral vaccination of Balb/C mice and determine the mechanism of immune induction.

**Methods:** Immune response was assessed by MPER-specific IgG serum ELISA and IgA ELISPOT. To determine the PP rLA uptake and antigen presenting cell (APC) trafficking to mesenteric lymph nodes (MLN), rLA-MPER with and without FimH was injected into the intestines of anesthetized mice and PPs and MLNs collected for tissue bacterial quantification. Trafficking of rLA to the MLN was further evaluated by flow cytometry.

**Results:** Surface expressed FimH resulted in significant increases in MPER-specific serum IgG and IgA-secreting B cells. There was no difference in PP uptake between rLA-MPER with and without FimH. There was a significant increase in rLA-MPER+FimH trafficking to the MLN which was mediated by CD64<sup>+</sup> macrophages.

**Conclusions:** rLA-MPER surface-expressing FimH has the potential to be powerful mucosal vaccine platform as it induced an immune response to a weak immunogen. The mechanism of immune induction was mediated through trafficking of rLA-MPER+FimH to the MLNs and not through increased uptake into PPs.

#### **E-13: GRAFT VERSUS HOST DISEASE IN CD34+ HUMAN HEMATOPOIETIC STEM CELL TREATED NSG MICE**

Erica Twitchell, Shawna Jackman, Elizabeth Jackman, Danielle Brown

**Background:** Immunodeficient mice with humanized immune systems are a valuable tool to study the immunologic basis and treatment of numerous diseases. Graft versus host disease (GvHD) is a known complication of human allogeneic hematopoietic stem cell transplantation (HSCT). In an evaluation of proprietary-treated CD34<sup>+</sup> human stem cell safety, NOD scid gamma (NSG) mice developed lesions consistent with GvHD.

**Objective:** The objective of this study was to evaluate the safety of intravenously (IV) administered proprietary-treated CD34+ human stem cells.

**Methods:** Eight-to-ten-week old NSG mice were treated intraperitoneally with busulfan for myeloablation, 2 days prior to IV administration of proprietary-treated stem cells, non-treated stem cells, or vehicle.

**Results:** At day 170, several of the mice from both stem cell groups displayed lesions of GvHD including hepatic portal inflammation, and perfollicular and interface dermatitis. Lungs had perivascular mononuclear infiltrates and increased alveolar macrophages when compared to controls. Histiocytic infiltrates were in the bone marrow, spleen, and rarely mesenteric lymph nodes of animals from both treatment groups. Successful engraftment of the stem cells and establishment of a humanized immune system were supported by greater splenic weights, increased white pulp and splenic hematopoiesis, and increased cellularity of the thymus and mesenteric lymph nodes in treated versus control mice.

**Conclusions:** At the completion of this study, a subset of treated mice demonstrated lesions consistent with GvHD. It is important to be aware of the potential of this occurring in studies utilizing HSCT, and may serve as a model for GvHD in human recipients of allogeneic HSCT.

#### **E-14: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF ORGANS IN LUPUS-PRONE MOUSE STRAINS TREATED WITH SERPINB3**

Laura Cavicchioli, Elisa Pompermaier, Giuseppe Maggioni, Anna Ghirardello, Roberto Luisetto, Marianna Beggio, Andrea Doria

**Background:** Lupus-prone mouse strains, New Zealand Black/White (NZB/NZW F1) and Murphy Roth Large/*lpr* (MRL/*lpr*), are widely used in the study of different aspects of human systemic lupus erythematosus (SLE). In particular, MRL/*lpr* mice are known to develop autoimmune lymphoproliferative syndrome (ALPS). SERPINB3 is tested for therapeutic purposes and effective dose-response in lupus nephritis (LN).

**Methods:** 16 NZB/NZW F1 and 11 MRL/*lpr* mice were subdivided in groups and intraperitoneally injected with human recombinant SERPINB3 (7.5µg/0.1ml in PBS) or placebo in a prophylactic or therapeutic (NZB/NZW F1 only) approach for LN, starting from 17<sup>th</sup> week of age until spontaneous death. Among harvested organs from complete post mortem exam, spleen, salivary glands, lymph nodes were collected and FFPE slides were examined by HE stain and IHC for anti-CD3, anti-CD20, anti-PD-1, anti-PDL-1 markers.

**Results:** The histopathologic score system applied to the available spleens revealed a severe lymphoid follicular hyperplasia in 7 cases (5 NZB/NZW F1 and 2 MRL/*lpr* mice) and a neolymphomatous appearance in one NZB/NZW F1 mouse. Visceral and subcutaneous lymph nodes showed a mild (NZB/NZW F1) or moderate to severe (MRL/*lpr*) follicular hyperplasia. In 4/12 MRL/*lpr* mice cervical lymphoid masses (IHC

profile: *CD3+* *CD20-* *PDL-1+*), occasionally with hepatic or intestinal nodules, were detected.

**Conclusion:** the development of lymphoproliferative disorders in lupus-prone mouse strains is evidenced, especially in MRL/*lpr* mice, in particular with cervical (salivary) and visceral involvement. Current immunosuppressive therapies for ALPS primarily target autoimmune manifestations with non-specific or with variable success, thus highlighting the need for molecularly-targeted immunomodulatory treatments.

#### **E-15: EQUINE HERPESVIRUS TYPE 1 INDUCES A SEVERE NECROTIZING MENINGOENCEPHALITIS IN MICE**

Leonardo Mesquita, Rafael Costa, Laís Rodrigues, Eliana Villalobos, Maria do Carmo Lara, Enio Mori, Cláudia Mori, Paulo Maiorka

**Background:** The equine herpesvirus type 1 (EHV-1) is an emerging pathogen that causes myeloencephalopathy in horses. Although mouse models have been developed to understand EHV-1 pathogenesis, few EHV-1 strains have been identified as highly neurovirulent to mice.

**Objective:** The aim of this study was to evaluate the pathogenesis of two neurovirulent EHV-1 strains in mice.

**Methods:** C57BL/6 male mice (n = 4/group), 8-12 weeks-old, were intranasally infected with 25 µL of A4/72 and A9/92 ( $10^5$  TCID<sub>50</sub>/ml) EHV-1 strains. These mice were euthanized on 1, 2 and 3 days post-inoculation (dpi), and brains were collected for virus titration and histology. Control mice were inoculated with EMEM.

**Results:** Both EHV-1 strains reached the brain of infected mice on 2 dpi, with an increase in viral titers on 3 dpi, when EHV-1-infected mice exhibited a hunched posture, with seizures, and recumbency. Histologically, on 3 dpi, there was a severe and diffuse neuronal degeneration and necrosis with cavitation of adjacent neuroparenchyma on the olfactory bulb; pyriform cortex; olfactory tubercle; striatal area to rostral mesencephalon; and entorhinal area. In A4/72-infected mice, Virchow-Robin spaces from these areas were multifocally expanded by 1 to 4 layers of macrophages, and a lesser number of lymphocytes with rare neutrophils (perivascular cuffing). Many of these inflammatory cells also expanded the leptomeninges of A4/72-infected mice. In A9/92-infected mice, perivascular cuffing was less prominent.

**Conclusions:** Neurovirulent EHV-1 strains induced a fulminant necrotizing meningoencephalitis in mice. This model will be also useful to understand the mechanisms underlying extensive neuropathology induced by viral infections.

#### **E-16: SEQUENTIAL COLLECTION OF THE JUGULAR AND NODOSE VAGAL GANGLIA IN PIGS FOR STUDIES OF THE AUTONOMIC NERVOUS SYSTEM**

David Meyerholz, Leah Reznikov

Genetically-engineered pigs are commonly used to model human diseases. The autonomic nervous system has been increasingly associated in the pathogenesis or

exacerbation of several types of diseases. Therefore, investigating autonomic ganglia for markers of disease severity and identification of candidate targets for pharmacotherapeutics in pig models of human disease are of interest. Vagal ganglia are critical in the detection and modulation of sensory stimuli from numerous internal organs. Vagal ganglia are divided into two segments, the jugular (anterior) and nodose (posterior) ganglia that arise during development from neural crest and epibranchial placodes, respectively. These two ganglia are typically fused as one structure in mice, whereas, in larger species such as humans and pigs, these are reported as separate and distinct ganglia. Pigs (~5 months of both sexes) were studied as part of another IACUC approved project. At necropsy, a ventral midline incision was made over the larynx with the skin. Soft tissues were removed to the level of the vagus tract and near the cranial level of the larynx, the budding nodose ganglia (bilaterally) were consistently identified. The vagus nerve and nodose ganglia were left intact, but dissected free from adjacent soft tissues and the larynx and pluck removed. The vagus nerve was then dissected cranially from the nodose ganglia and within ~2-4 cm the jugular ganglia was located, near the exit site of the vagal nerve from the skull. Histologically, both ganglia were consistently identified and validated. These collection techniques will significantly aid scientific investigations in future studies.

#### **E-17: HISTOPATHOLOGICAL ANALYSIS ON DISTRIBUTION OF NEURONAL DAMAGE AND GLIAL CELL REACTION CAUSED BY TRANSIENT HYPOGLYCEMIA IN RAT**

Nagi Tomita, Yuji Sunden

**Background:** The previous animal models of hypoglycemic brain damage have been given long time hypoglycemia to evoke severe neuronal damage, but it was too severe to detect difference of the neuronal vulnerability in the brain in detail. Pathogenesis of the brain damage remains unclear, but a recent study demonstrated that microglial cell activation might be responsible for hypoglycemic neuronal death [Won SJ *et al*, 2012].

**Objective:** To reveal the distribution of the neuronal death and glial cell reaction after hypoglycemia in detail by using transient-hypoglycemic rats.

**Methods:** Rats were given insulin-induced hypoglycemia (10-30 min) and recovered. Brains were collected temporally and submitted for histopathological and immunohistochemical (IHC) analysis (antibodies; Iba-1, GFAP, NeuN, calbindin).

**Results:** In the cerebral neocortex layer 2-3, diffuse neuronal death was seen at 1-30 days post hypoglycemia (dph). NeuN IHC revealed that neuronal loss was more significant in layer 3 than that in layer 2 and that a part of the parietal cortex was preserved. In the neocortex layer 1-3, the number of Iba-1 positive microglial cells and GFAP positive astrocytes diffusely increased. In the cingulate, these findings were not recognized. In the cerebellum, calbindin IHC showed that scattered loss of Purkinje cells was mostly seen in the surface of cerebellar folia adjacent to the primary fissure at 3 -14 dph.

**Conclusions:** The cerebral neocortex showed neuronal death and glial cell reaction after hypoglycemia, while the cingulate was preserved. In cerebellum, delayed Purkinje cell loss might occur at a few days after hypoglycemia.

#### **E-18: DISTRIBUTION OF ATYPICAL PORCINE PESTIVIRUS IN THE CEREBELLUM OF PIGS FOLLOWING IN UTERO INOCULATION**

Shollie Falkenberg, Paulo Arruda, Drew Magstadt, Kent Schwartz, Franco Matias Ferreyra, Mitchell Palmer, Justin Greenlee, John Neill, Igor Gatto, Julia Ridpath, Bailey Arruda

**Background:** Atypical porcine pestivirus (APPV) is a cause of congenital tremors (CT), but APPV associated CT resolves over time. Clinical evidence implicates the presence of an underlying abnormality of cerebellar function as CT is characterized as an intention (i.e., “cerebellar”) tremor.

**Objective:** The aim of this study was to evaluate viral distribution of APPV in the cerebellum and the potential implications.

**Methods:** Piglets infected *in utero* with APPV or PBS were euthanized at 2 days of age and evaluated for viral localization by ISH. Boars infected *in utero* with APPV were maintained after resolution of CT and euthanized at approximately 11 months. RNAscope<sup>®</sup> probes were designed for the N<sup>pro</sup>-E<sup>ns</sup> coding region of APPV.

**Results:** In piglets, viral RNA had multifocal distribution within granular layers that extended into the adjacent molecular layer. The degree of labeling varied between animals, ranging from one to several small foci to larger locally extensive areas. In the boars, extensive labeling of the molecular and granular layers was observed with minimal to no areas that lacked viral labeling.

**Conclusions:** This data highlights the inconsistency in the distribution of the virus in the cerebellum of piglets and extensive labeling observed in boars in which CT had resolved. Investigations elucidating the cascade of biochemical and cellular events occurring in the cerebellum in CT as well as the physiological effects of secondary remodeling/rewiring that may be occurring in piglets with CT that result in resolution of clinical disease despite persistent infection of cells in the cerebellum warrants investigation.

#### **E-19: AGING-LIKE SPONTANEOUS EPIGENETIC SILENCING FACILITATES WNT ACTIVATION, STEMNESS AND BRAFV600E-INDUCED TUMORIGENESIS**

Byunghak Kang, Yong Tao, Daniel Petkovich, Julie In, Genevieve Stein-O'Brien, Xiangqian Kong, Wenbing Xie, Nicholas Zachos, Shinji Maegawa, Ray-Whay Yen, Elana Fertig, Jean-Pierre Issa, Stephen Baylin, Hariharan Easwaran

**Background:** Spontaneous promoter DNA hypermethylation associated gene silencing arises during aging and in early carcinogenesis, yet its role in early carcinogenesis is underexplored. An important scenario is Human *Braf*-V600E-mutant colon adenocarcinomas (COAD), which harbor abnormal promoter CpG-island methylator

phenotype (CIMP). Understanding mechanisms of CIMP and oncogenic-BRAF interaction in COAD initiation could lead to useful clinical interventions.

**Objectives:** To characterize the spontaneous promoter DNA hypermethylation in mouse colon-derived organoids and its role in carcinogenesis of COAD in the context of *Braf*-V600E mutation.

**Methods:** Organoids were prepared from the proximal colon of heterozygous cre-inducible *Braf*V600E (*Braf*<sup>+/LSL</sup>) and wild-type siblings. The organoids were cultured in stem cell niche factor enriched media. Their phenotypes were analyzed at different time points using Wnt dependency assays, xenograft assays, DNA methylation and expression profiling.

**Results:** The spontaneous promoter DNA hypermethylation in mouse colon-derived organoids mimics the human “aging-like” methylation profile, and associated gene silencing promotes activation of the Wnt pathway inducing an autonomous stem-like proliferation and dedifferentiation of colon-derived organoids. These changes render aged organoids profoundly sensitive to oncogenic transformation by *Braf*-V600E mutation. Consistent with this result, CRISPR-mediated simultaneous inactivation of a panel of the silenced genes markedly sensitizes the organoids to *Braf*-V600E-induced transformation.

**Conclusions:** Spontaneous promoter DNA hypermethylation in mouse colon-derived organoids promotes autonomous stem-like proliferation and dedifferentiation, which facilitates *Braf*-V600E mutation to drive tumor initiation. Our studies underscore the importance of age-related epigenetic abnormalities in modulating stem cell states and predisposing to tumor induction upon acquisition of oncogenic mutations.

## **E-20: CHARACTERIZATION OF BLOOD-TUMOR BARRIER PATHOLOGY IN A MOUSE MODEL OF BRAIN METASTASES OF LUNG CANCER**

Alexandra Dieterly, Gozde Uzunalli, Chinyere Kemet, Arvin Soepriatna, Craig Goergen, Michael Wendt, Tiffany Lyle

The incidence of brain metastases of lung cancer is increasing. Current treatment of brain metastases is ineffective due to poor uptake and the transformation of the blood-brain barrier (BBB) into the blood-tumor barrier (BTB). We identified altered functional components of the BBB in a mouse model non-small cell lung cancer (NSCLC) brain metastases. A brain-seeking variant of NSCLC was injected into the left cardiac ventricle of nu/nu mice and brains were harvested within six-weeks. In each mouse the BBB and BTB were qualitatively and quantitatively analyzed using immunofluorescence microscopy. We report a 5.4-fold decrease in CD31 (endothelial cell) expression in the BTB compared to the BBB. A 1.7-fold decrease in the tight junction adapter protein, zona occludens-1 was present, in the BTB compared to the BBB. The parenchymal basement membrane was altered in the BTB, exhibiting a 7.4 fold decrease in laminin- $\alpha$ 2 expression. A loss of multiple pericyte subtypes was identified in the BTB. We identified a 2.6-fold decrease in expression of PDGFR- $\beta$ , a pan-pericyte protein, within the BTB. There was a 7.5-fold decrease in resting pericytes and a 2.0-fold decrease in

contractile pericytes. Similar pathologic changes were identified in the BTB at five weeks post-intracardiac injection. Our findings suggest that the tight junction adaptor protein, parenchymal basement membrane, and pericytes are critical in maintaining physiologic integrity of the BBB. A distinct set of BBB alterations occur in the brain metastases of lung cancer, and tight junctions, basement membranes, and pericytes may be effective chemotherapeutic targets to improve patient survival.

## **E-21: CONSEQUENCES OF PHYSIOLOGICAL SALINE INJECTION INTO THE MURINE SPINAL CORD ON MORPHOLOGICAL AND MOLECULAR LEVEL**

Laura Zind, Martin Gamber, Katja Matheis, Arno Kalkuhl, Ulrich Deschl, Wolfgang Baumgärtner, Florian Hansmann

**Background:** Intervention strategies in spontaneously occurring diseases and animal models for CNS diseases frequently include injections of drugs and/or cells in the central nervous system. The treatment related effects may be obscured by various factors including the injection itself.

**Objective:** The aim of the present study was to investigate the effects of physiological saline injection in the murine spinal cord on morphological and molecular level.

**Methods:** Eight-week old, female C57Bl/6 mice received an intraspinal injection of physiological saline. Age and sex matched, untreated C57Bl/6 mice served as controls. Mice were clinically investigated in an open field using BMS scale. Necropsies were performed at 3, 21, 63 and 126 days post injection (dpi). Spinal cord was investigated using histology, immunohistochemistry targeting lymphocytes, microglia/macrophages, astrocytes, axonal damage and demyelination. Additionally, transcriptome analysis was performed using RNA sequencing.

**Results:** Clinical investigation revealed no significant differences between injected and control mice at all dpi. An increased number of npNF labeled axons was detected in injected mice at 3 and 21 dpi while no significant inflammation, astrogliosis or demyelination was detected. Transcriptome analysis revealed 2091, 431, 10 and 24 differentially expressed genes (DEGs) at 3, 21, 63 and 126 dpi, respectively. Interestingly, the majority of genes were upregulated. Important canonical pathways at early time points include granulocyte adhesion and diapedesis and acute phase signaling.

**Conclusion:** Intraspinal physiological saline injection resulted in a significant and timely progressively decreasing number of DEGs, which was not associated with the occurrence of clinical signs.

## **E-22: SPONTANEOUS BACKGROUND BRAIN PATHOLOGY IN C57BL/6J MICE**

Jimmy Tarrant, Lorna Omodho, Patrick Savickas, Marco Spinazzi, Enrico Radaelli

**Background:** Definition of the background or incidental lesions associated with a specific mouse strain is vital to correct interpretation of pathological endpoints in the experimental context. The C57BL/6J inbred strain represents the reference congenic background to which most genetically engineered mutations (GEM) are backcrossed.

GEM on a C57BL/6J background are widely employed as models to study brain disorders in humans. However, comprehensive studies detailing the spontaneous brain pathology in C57BL/6J mice are lacking.

**Objective:** To define the nature and frequency of spontaneous neuropathological changes on a large cohort of C57BL/6J mice and their association with specific biological variables.

**Methods:** Brains from 183 experimentally naïve and clinically unremarkable C57BL/6J mice were collected and processed routinely for histopathology. Mice ranged in age from 2 to 110 weeks with 74 females, 106 males, and 3 unknown. Coronal sections of at least five levels were examined for each individual using the following anatomical landmarks: paraflocculi, mammillary body, caudal aspect of the optic chiasm, and olfactory tubercles.

**Results:** Sixteen spontaneous findings were described. Age-related neurodegenerative changes represented the most common pathologic changes and included: (i) Hirano-like inclusions in the thalamic neurons, (ii) neuroaxonal dystrophy in the medulla oblongata, (iii) PAS-positive granular deposits in the neuropil of the hippocampus. Neoplastic and inflammatory lesions were rarely observed.

**Conclusions:** This work provides a detailed documentation of the nature and frequency of spontaneous brain lesions of C57BL/6J mice representing an invaluable reference for the interpretation of lesions found in GEM models harboring mutations contributing to neuropathology.

### **E-23: IMPAIRED SPERMATOGENESIS IN PARL-DEFICIENT MICE**

Enrico Radaelli, Lorna Omodho, Patrick Savickas, Marco Spinazzi

PARL is a rhomboid family intra-membrane protease present in the inner mitochondrial membrane. While PARL was originally described as an antiapoptotic protein, its role in mitochondrial homeostasis and the molecular mechanism underlying the clinical phenotype are currently unclear. *Parl*-deficient mice die soon after puberty because of the development of a severe progressive multisystemic disease characterized by cachexia and locomotor impairment. Severe testicular degeneration and atrophy represent one of the earliest manifestation invariably observed in male *Parl*-deficient mice. Pathological changes diffusely affect seminiferous tubules that show pre-meiotic spermatogenesis arrest at the level of primary spermatocytes. Impaired spermatogenesis is also accompanied by intense exfoliation of degenerated giant multinucleated spermatocytes into the tubular lumen. These lesions appear to be associated with severe depletion of the mitochondrial GPX4 isoform, nuclear accumulation of the cellular tumor antigen p53 but no evidence of apoptosis. Mitochondrial GPX4 and p53 levels in other *Parl*-deficient tissues (including brain, liver, lymphoid organs and skeletal muscle) were not changed. Interestingly, mitochondrial GPX4 and p53 are well-documented negative and positive regulator of ferroptosis, respectively. Altogether, this evidence suggests a potential tissue-specific role for PARL

in the mechanisms that maintain spermatogenesis via mitochondrial GPX4 and regulation of ferroptosis.

#### **E-24: REDUCED PLACENTAL EXPRESSION OF REGULATOR OF G PROTEIN SIGNALING-2 (RGS2) IN A MOUSE MODEL IS SUFFICIENT TO INDUCE HISTOPATHOLOGIC AND TRANSCRIPTOMIC CHANGES TYPICAL OF PREECLAMPSIA**

Katherine Gibson-Corley, Katherine Perschbacher, Guorui Deng, Eric Devor, Gary Pierce, Rory Fisher, Donna Santillan, Mark Santillan, Justin Grobe

**Background:** Preeclampsia (PreE), a cardiovascular disorder of pregnancy, remains a major cause of maternal and fetal mortality worldwide. Mutations of Regulator of G protein signaling-2 (*RGS2*) have been associated with increased risk for PreE in humans. We demonstrated that breeding C57BL/6J dams with *Rgs2*-deficient (*Rgs2*-KO; B6.129P2-*Rgs2*<sup>tm1Dgen</sup>/Mmnc) sires leads to reduced placental expression of *Rgs2* mRNA and hypertension and proteinuria typical of PreE in dams.

**Objective:** Our objective was to determine whether paternal contribution of an *Rgs2*-null allele is sufficient to cause histopathologic and molecular changes within the mouse placenta that are described in PreE.

**Methods:** Histopathologic analyses and RNA sequencing were performed on placentas collected on gestational day 12.5 from C57BL/6J dams mated with either *Rgs2*-KO sires or wildtype littermates of these sires.

**Results:** We have identified that placentas from dams mated with an *Rgs2*-KO sire have decreased spiral artery number and diameter as well as decreased CD31 immunoreactivity in all placental layers, which have been described in placentas from human PreE patients. RNA seq analysis of placentas from these mice identified differential expression of 726 genes (479 up, 247 down), including *Hsd11b2* (up) and *Adm* (down), which are similarly changed in human PreE placenta. Reduced placental *Rgs2* was associated with gene signatures suggesting mitochondrial dysfunction, unfolded protein response, and oxidative stress, also paralleling findings in human PreE placenta.

**Conclusions:** Collectively these data support the conclusion that reduced *Rgs2* expression in the feto-placental unit is sufficient to induce histopathologic and transcriptomic changes within the placenta that are typical of PreE.

#### **E-25: THERAPEUTIC EFFECT OF THE HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV) REPLICATION INHIBITOR JNJ-64166037 IN HRSV INFECTED LAMBS**

Panchan Sitthicharoenchai, Mark Ackermann, Sarhad Alnajjar, Alejandro Larios-Mora, David Lançois, Peter Rigaux, Dirk Roymans, Jack Gallup

**Background:** Human respiratory syncytial virus (hRSV) is a major cause of viral respiratory tract infection in infants resulting in bronchiolitis and pneumonia. A neonatal lamb model of hRSV infection has been established to study the disease mechanisms and response to treatment.

**Objective:** The aim of this study was to evaluate the efficacy of a small molecule replication inhibitor, JNJ-64166037, for treatment of hRSV by experimental trial in the hRSV infected lamb model.

**Methods:** Neonatal lambs were inoculated with hRSV Memphis 37 via nebulization. Subsequent to viral inoculation, lambs were treated orally 1 hour after the infection, then once daily with a dose range of antiviral compound (2, 10 and 50 mg/kg). At day 6 post-infection, lung lesions were evaluated and samples were collected from infected animals to determine the therapeutic outcome.

**Results:** A dose-dependent antiviral effect was observed with reduction (>100 fold) of the detectable lung infectious virions at 50 mg/kg. Consistently, the amount of viral antigen detected in the lung was significantly lower at 50 mg/kg and elevated with lower dose of treatment. Similar trends were observed in bronchoalveolar lavage samples. Lack of macroscopic and microscopic lung lesions were observed with this dose regimen.

**Conclusion:** Oral administration of JNJ-64166037 at the appropriate dose has antiviral effects in the lamb model of RSV infection. This mode of therapeutic intervention eliminated the hRSV-induced lung lesions assessed in the model. JNJ-64166037 might offer new opportunities of RSV bronchiolitis treatment in infants and children.

## **E-26: THE ROLE OF SREBP-SCAP IN PANCREATIC DUCTAL ADENOCARCINOMA**

Stephanie Myers, Meredith McGuire, Wei Shao, Theodore Ewachiw, Zeshaan Rasheed, Peter Espenshade

**Background:** Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive tumor with few diagnostic and therapeutic options. Due to its highly proliferative nature, PDAC tumor cells have a high demand for lipid synthesis. However, this tumor is known to be poorly vascularized and nestled within a hypoxic environment. As lipid synthesis is a highly oxygen-consumptive process, neoplastic cells are challenged with meeting the demand for lipids. In particular, the SREBP-SCAP pathway is one of interest in lipid sensing and synthesis.

**Objectives:** Our objective was to determine the role of the SREBP-SCAP pathway in PDAC cell growth during lipid-poor conditions *in vitro* as well as *in vivo* using mouse models.

**Methods:** Four patient-derived pancreatic adenocarcinoma cell lines were utilized. *SCAP* was knocked out, then rescued in all cell lines. Subcutaneous tumor xenografts were performed in nude mice using both wildtype and *SCAP* knockout cells. Functional growth assays were performed using wildtype, *SCAP* knockout, and *SCAP* rescued cell lines in both lipid-poor and lipid-rich conditions. A known mouse line, *LSL-Trp53<sup>R172H</sup>; LSL-Kras<sup>G12D</sup>; Pdx-1 Cre* (KPC), is utilized as a PDAC model.

**Results:** In tumor xenografts, *SCAP* knockout cells showed slower growth of tumor volume in 3 out of 4 cell lines. In functional assays, *SCAP* knockout cells exhibited significantly reduced growth in lipid-poor conditions. KPC mice effectively form PDAC.

**Conclusions:** The specific loss of SCAP in PDAC tumor cells alters the growth capability both *in vitro* and *in vivo*. Further studies are needed to elucidate the specific mechanism of altered cell growth.

#### **E-27: DEER MICE AS AN ANIMAL MODEL FOR INFECTION, PATHOGENESIS, AND IMMUNITY OF HEPATITIS C-LIKE VIRUSES**

Olivia Swartley, Sashi Rekha Gadi, Amit Kapoor, John Cullen

**Background:** Approximately 71 million people are chronicity infected with Hepatitis C virus (HCV). HCV has a narrow host range that is mostly limited to humans and chimpanzees. The lack of a tractable small animal model has limited extensive virus-host studies until 2013 when homologs of HCV were discovered in wild rodents.

**Objective:** To describe the biological characterization of a new rodent hepacivirus (RHVpl-A1) that successfully infects deer mice and mirrors HCV in its biological properties.

**Methods:** We used liver samples from 140 *Peromyscus* species (*P. maniculatus*, *P. leucopus*, and *P. californicus*) to find seven different rodent hepaciviruses variants. Pairs of seronegative mice were inoculated with the each virus. We selected 1 virus (named RHV-pl-A1) for subsequent studies as it showed hepatotropism as well as persistent and self-limited infection. 60 seronegative mice were infected and followed for viremia and IgG titers using serology and PCR. Histology confirmed hepatotropism.

**Results:** 50 of the RHV-pl-A1 mice were able to be followed for 8 months. Similar to HCV infected humans, we observed different infection outcomes. 42 mice had a definitive outcome of either spontaneous virus clearance or persistent viremia, 4 mice remained uninfected, and 4 mice exhibited viremia but had no antibody response. Of the 42 mice with definitive results, 21 cleared the infection and 21 remained chronically infected.

**Conclusions:** RHV-pl-A1 infected deer mice are tractable and fully immunocompetent animal models for hepatitis C-like viruses with which the mechanism of hepacivirus infection, clearance, and persistence can be pursued.

#### **E-28: PREVENTIVE ANTAGONISM OF THE AR SIGNALING AXIS INHIBITS HEPATOCELLULAR CARCINOGENESIS IN A RAT MODEL**

Timothy Helms, Jennifer Thomas-Ahner, Moray Campbell, Steven Clinton, Christopher Coss

Early castration (ORX) and lifelong knockout of the hepatic androgen receptor (AR) inhibit hepatocellular carcinogenesis in rodent models. We hypothesized that early pharmacologic antagonism of the AR would similarly inhibit carcinogenesis. To test this hypothesis and elucidate AR's role in HCC, we preventively administered enzalutamide (ENZ), a potent non-steroidal AR antagonist, to 6-week old male rats before inducing carcinogenesis via diethylnitrosamine (DEN) and partial hepatectomy. We identified that 30 mg/kg ENZ decreased the number of GST-P positive, pre-neoplastic foci compared to untreated controls. This effect occurs in the absence of hepatic AR at the time of

challenge. AR is upregulated 4-weeks following challenge; preliminary evidence attribute this effect to enhanced cytoplasmic expression in atypical hepatocytes. Microarray analysis of ENZ treated livers identifies selective preservation of periportal cholesterol and lipid metabolism gene expression compared to untreated controls while ORX sustains perivenous zinc/transition metal homeostasis gene expression along with CYP7A1 and CYP2E1. Preventive ENZ and ORX also differentially mitigate chronic DEN mediated STAT3 activation where ENZ downregulates STAT3 phosphorylation while ORX inhibits its expression.

**Conclusions:** Low dose, preventive antagonism of the AR inhibits hepatocellular carcinogenesis.

Hepatic AR, while not detected at challenge, is upregulated early in HCC.

ENZ and ORX modulate expression of zonally restricted genes implicating an androgen influence on hepatic metabolic zonation.

ENZ and ORX inhibition of STAT3 activation/expression, respectively, suggest an impact of androgens on this carcinogenic pathway.

Androgen deprivation therapies via ligand deprivation (ORX) and receptor antagonism (ENZ) are functionally unique in their inhibition of HCC.

## **E-29: EXPLORING PHARMACOLOGICAL ALTERNATIVES AGAINST CLOSTRIDIUM PERFRINGENS ENTEROTOXIN IN VIVO**

Mauricio Navarro, John Freedman, Eleonora Morrell, Juliann Beingesser, Archana Shrestha, Bruce McClane, Francisco Uzal

**Background:** *Clostridium perfringens* enterotoxin (CPE) is a pore-forming toxin responsible for several gastrointestinal diseases in humans, including enterotoxemic death associated with hyperpotassemia. CPE cytotoxicity in cultured enterocyte-like cells involves caspase-3 activation and apoptosis with low CPE dose and necrosis with high CPE dose. The acridine-derivative mepacrine reduces cytotoxicity by inhibiting CPE-pore formation and activity *in vitro*.

**Objective:** To explore novel pharmacological targets against CPE-related diseases. Two hypotheses were tested: i) CPE-induced caspase-3 activation occurs in enterocytes and it can be inhibited *in vivo* and ii) mepacrine prevents CPE-induced intestinal disease and enterotoxemia.

**Methods:** Purified CPE was inoculated into ligated small intestinal loops of Balb/c mice in increasing concentrations for 2 hr. Then, groups of mice received CPE (100µg) plus a pan-caspase inhibitor (25µg) or one of two concentrations of mepacrine (0.5 or 1.0mM). Survival was recorded until 4 hr post-challenge. Intestinal samples were collected for histopathology and activated-caspase-3 quantitation. Serum samples were collected for CPE detection and potassium measurement.

**Results:** CPE induced intestinal damage and caspase-3 activation in enterocytes, dose-dependently. A pan-caspase inhibitor significantly reduced caspase-3 activation; however, this inhibition did not protect mice from intestinal damage or enterotoxemic lethality. Mepacrine protected mice from CPE-induced intestinal damage and enterotoxemia; this protection involved reduction of CPE-induced hyperpotassemia. Intestinal CPE absorption was not significantly reduced.

**Conclusions:** i) CPE-induced caspase-3 activation occurs in enterocytes *in vivo*, but this activation is not essential to cause intestinal damage or enterotoxemia; ii) mepacrine is a potential therapeutic alternative against intestinal disease and enterotoxemia caused by CPE.

### **E-30: IMMUNOPHENOTYPING OF LYMPHOCYTES IN A MINIPIG MODEL OF VASCULARIZED COMPOSITE ALLOTRANSPLANTATION REJECTION**

Caitlin Mason, Georg Furtmuller, Gerald Brandacher, Sarah Beck

Vascularized composite allotransplants (VCAs) are compound transplants that include multiple distinct tissue types, including skin, muscle and other associated tissues. With rigorous immunosuppressive therapy, successful transplantation can be maintained in both animal models and human patients with VCAs with low rates of acute rejection. Clinically, the main target of rejection appears to be the skin, and although the exact immunologic mechanism remains unclear, clinicopathologic monitoring of the skin provides a useful metric for the rejection status of the graft as a whole. A stifle VCA rejection model is achieved with MHC class 1 and 2-mismatched Massachusetts General Hospital minipigs, a model with similar dermohistomorphology to humans. However, more detailed studies of the pathology of porcine dermal tissues in acute VCA rejection are necessary. The aims of this project include developing a scoring system based on the human system of the dermatohistopathology of acute rejection in the minipig model and characterizing the lymphocytic infiltrate of the skin in VCA rejection controls using immunohistochemical staining. The overarching goals of IHC analysis include optimization of CD3+, CD20+, and FoxP3+ primary antibodies in control lymphoid tissues, and quantification of the amount of CD3+, CD20+, or FoxP3+ inflammation in acute graft rejection. Based on observations in human VCA rejection, we hypothesize that the inflammation will be predominantly CD3+ T cells with significantly fewer CD20+ B cells and FoxP3+ regulatory T cells. These studies will set the groundwork for future immunologic characterization of VCA rejection using the MGH minipig model under a variety of immunomodulatory therapies.

### **E-31: EQUINE HOOF LAMELLAR STRUCTURAL FAILURE IS PREVENTED BY CONTINUOUS DIGITAL HYPOTHERMIA IN A MODEL OF ENDOCRINOPATHIC LAMINITIS**

Simon Stokes, James Belknap, Andrew van Eps, Julie Engiles, Francois Bertin, Carlos Medina-Torres

**Background:** Continuous digital hypothermia (CDH) can prevent lamellar structural failure in sepsis-associated laminitis and has led to a better understanding of the

pathophysiology of that form of laminitis, however the effects of CDH in endocrinopathic laminitis are unknown.

**Objective:** To determine if CDH prevents lamellar structural failure in the euglycemic hyperinsulinemic clamp (EHC) model of endocrinopathic laminitis.

**Methods:** Eight clinically normal Standardbred horses underwent laminitis induction using the EHC model. At initiation of the EHC, one forelimb was randomly selected to be continuously cooled (ICE), with the other maintained at ambient temperature (AMB). Dorsal lamellar sections (proximal, middle, distal) were harvested 48 h after initiation of the EHC and were analysed using histological scoring and histomorphometry. Cell proliferation was quantified by counting epidermal cell nuclei staining positive for an immunohistochemical proliferation marker (TPX2).

**Results:** Median [interquartile range] histological scores were greater ( $P<0.05$ ) in AMB (proximal 3 [3-3]; middle 3 [3-3]; distal 3 [3-3]) compared with ICE limbs (proximal 2 [1.25-2]; middle 1 [1-2]; distal 1 [1-2]). Severe dermo-epidermal separation was observed in all AMB feet at one or more section levels, which was not observed in ICE sections. Histomorphometry measurements of total and non-keratinised primary epidermal lamellar length were significantly increased ( $P<0.05$ ) in AMB limbs compared to ICE limbs at all 3 levels. TPX2 positive cell counts were significantly increased ( $P<0.05$ ) in AMB limbs compared to ICE limbs.

**Conclusions:** CDH reduced the severity of lamellar histological lesions in the EHC model and prevented lamellar structural failure (dermo-epidermal separation).

## **E-32: DEVELOPMENT OF A PORCINE MODEL FOR CARBON PARTICLE MIGRATION TO REGIONAL LYMPH NODES**

Raffaele Melidone, David Spenciner, Alan Barber, Brett Zani, Timothy Muench

**Background:** Intra-articular wear particulate migration from the knee joint has been studied in various animal models, as well as post-mortem in patients who received total knee joint replacement. However, there still exists a need for a simple yet analogous animal model for tracking the migration of wear debris from the knee joint, especially through the draining lymph nodes.

**Objective:** To fill this need, a proof-of-concept porcine model was developed for particle migration from the knee joint into the surrounding lymphatic system.

**Method:** Vitreous carbon particles were deposited both intra-articularly and extra-capsularly in a bilateral fashion to the hind limbs in 6 Yorkshire swine. The regional/draining lymph nodes were qualitatively assessed weekly by a veterinarian via manual palpation to detect any enlargement or change in consistency when compared to the initial assessment prior to the surgical procedure. At 6 weeks, the draining lymph nodes were harvested and processed for histology.

**Results:** At 6 weeks, the draining lymph nodes were harvested and processed for histology. Microscopic evaluation revealed carbon particle migration from the knee into

iliac lymph nodes (12 of 12) and inguinal lymph nodes (6 of 12), but no evidence of particle migration into popliteal lymph nodes. Particle associated inflammation was generally minimal, consisting of neutrophils, histiocytes, multinucleated giant cells, and eosinophils.

**Conclusions:** Overall, this study established a needed animal model for evaluating particle migration to the draining lymph nodes from the knee joint.

### **E-33: DEFINING THE HOST RANGE OF AQUATIC BIRD BORNAVIRUS THROUGH GROWTH AND LESION DEVELOPMENT IN OVO**

Alex Leacy, Eva Nagy, Nicole Nemeth, Csaba Varga, Leonardo Susta

Aquatic bird bornavirus (ABBV) is the causative agent of a chronic and debilitating ganglioneuritis and encephalitis. ABBV is highly prevalent in migratory waterfowl but has been sporadically isolated from other birds including gulls, bald eagles, and emus, suggesting a broad host range. Given the ability to infect multiple species, ABBV may have the potential to infect commercial poultry (i.e., chickens and turkeys). In this study, we aimed to evaluate the host restriction of ABBV through growth and lesion development *in ovo*. A strain of ABBV was isolated from a Canada goose and propagated in primary goose embryo fibroblasts to create stock virus. Groups of duck, chicken and turkey eggs were inoculated with ABBV into the yolk sac (day 6 of incubation for chickens; day 8 of incubation for turkeys and ducks) or into the allantoic cavity (day 9 of incubation for chickens; day 12 of incubation for turkeys and ducks). Embryonic tissue was harvested at two time points during the experiment—early (day 12 of incubation for chickens; day 15 of incubation for turkeys and ducks) and late (day 19 of incubation for chickens; day 24 of incubation for turkeys and ducks). Virus growth was evaluated through qRT-PCR of embryonic tissues (brain, heart, liver, proventriculus/ventriculus, chorioallantoic membrane, yolk and allantoic fluid) and lesion development was assessed through histopathology. Results showed that growth in embryonic tissue was limited and lesion development was absent. This research is the first to evaluate an *in ovo* model to assess ABBV host restriction.

### **E-34: RECOMBINANT HEMAGGLUTININ GLYCOPROTEINS DERIVED FROM HIGHLY PATHOGENIC AND LOW PATHOGENIC H5 AVIAN INFLUENZA VIRUSES REFLECTS TISSUE TROPISM AND HOST SUSCEPTIBILITY ACROSS AVIAN SPECIES**

Carmen Jerry, Christina Leyson, David Stallknecht, Monique Franca

**Background:** The Avian Influenza Virus (AIV) hemagglutinin (HA) is a major determinant of viral attachment and infection. Evaluation of the HA tropism may explain differences in infectivity and pathogenesis of AIV across avian species.

**Objective:** To compare the tissue tropism of the H5 HA, focusing on respiratory and gastrointestinal tissues from wild and domestic birds, using recombinant HA protein with gene sequences derived from low pathogenic (LPAIV) and highly pathogenic (HPAIV) H5 AIV isolates.

**Methods:** The HA of A/Northernpintail/Washington/40964/2014(H5N2) (highly pathogenic AIV) and A/mallard/MN/410/2000(H5N2) (low pathogenic AIV) were codon optimized and cloned into mammalian expression vectors, then transfected into HEK 293T cells to produce the recombinant HA glycoproteins. Representative species from orders *Galliformes*, *Passeriformes*, *Anseriformes*, *Charadriiformes*, *Accipitriformes* and *Columbiformes* were used. The amount of HA binding in tissues was assessed using ImageJ2 software.

**Results:** Significant binding of the HP and LP H5 HA was noted in the trachea and lung of species of the order *Anseriformes*. In the intestinal tract, HA binding was localized to enterocytes and goblet cells of the cecum, with both H5 HA proteins. The small intestine of most species lacked binding of both HA proteins, however, minimal binding was seen in *Anseriformes* and *Galliformes* using the HP H5 HA. Cloacal bursas had mild binding in *Anseriformes* and *Galliformes* species.

**Conclusions:** Investigation of recombinant HP and LP H5 HA binding across avian species showed that respiratory and intestinal tissues from birds of the orders *Anseriformes* had the most significant binding, followed by *Charadriiformes*, and *Galliformes*.

#### **E-35: DISEASE PHENOTYPE OF A SHEEP MODEL OF CYSTIC FIBROSIS GENERATED BY CRISPR/CAS9 DISRUPTION OF THE CFTR GENE**

Arnaud Van Wettere, Zhiqiang Fan, Luri Viotti Perisse, Calvin Cotton, Misha Regouski, Qinggang Meng, Zhongde Wang, Ann Harris, Kenneth White, Irina Polejaeva

**Background:** Cystic Fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The major cause of limited life-span in CF patients is progressive lung disease. Several CF models (mice, rats, ferrets, and pigs) have been generated to enhance our understanding of the CF pathogenesis.

**Objective:** Sheep may be a particularly relevant animal to model CF in humans due to the similarities in lung anatomy and development in the two species. Here, we characterized the phenotype of the first sheep model of CF.

**Methods:** Knocked-out *CFTR* cell lines were created using CRISPR/Cas9 technology and Somatic Cell Nuclear Transfer (SCNT) techniques were used for production of *CFTR*<sup>-/-</sup> and *CFTR*<sup>+/-</sup> lambs. Necropsy and histopathology were used to document the morphologic changes.

**Results:** The newborn *CFTR*<sup>-/-</sup> sheep develops disease consistent with CF pathology in humans but with a more severe phenotype including intestinal obstruction similar to meconium ileus, absence of vas deferens and pancreatic fibrosis. Additionally, hepatic portal fibrosis and biliary hyperplasia resembling biliary fibrosis, cholestasis, and gallbladder hypoplasia that may reflect advanced CF disease in humans were present.

**Conclusion:** *CFTR*<sup>-/-</sup> sheep show several abnormalities seen in human newborn with CF. Though, it is too early to know whether the CF sheep will recapitulate human CF

lung disease over time, this model will likely be a useful resource to advance the development of new CF therapeutics.

### **E-36: SYNERGISTIC ACTION OF ONCOLYTIC VIROTHERAPY WITH NEWCASTLE DISEASE VIRUS AND PACLITAXEL ON CHEMICALLY-INDUCED MAMMARY CANCER IN RATS**

Sahar Abd El-Rahman, Azza Hassan, Amani Saleh, Khodeir H, Alaa Abd El-Khalek

**Background:** Virotherapy is a very promising treatment modality, as it is selective, safe, and causes cancer destruction. Newcastle disease virus(NDV) has been applied to oncolytic virotherapy for decades due to its naturally oncolytic property.

**Objective:** The current study aimed to focus on the potential oncolytic effect of Newcastle disease virus on 7,12 Dimethyl-benzanthracene(DMBA) -induced mammary cancer in rats. All experiments using animals were performed according to the protocol approved by the Institutional Animal Care and Use Committee at Cairo University(IACUC).

**Methods:** For this reason, therapy was started in 3 groups of DMBA-induced mammary cancer in virgin female rats, using the lentogenic LaSota strain of NDV ( $10^9$  TCID<sub>50</sub>)i/p, paclitaxel (5mg/kg b.wt)i/p and their combination. A group of non-tumor-bearing rats were injected with the LaSota strain of NDV.

**Results:** It was found that LaSota strain of NDV exhibited significant chemotherapeutic effect through several pathways including significant tumor regression by decreasing the size and number of tumors/ rat with marked tumor necrosis, significant amelioration of the oxidative stress markers' alterations as well as significant inhibition of CD44, VEGF, Ki-67 and MMP-2 expression associated with significant increased expression of Beclin-1 and LC3. In addition, the combination of LaSota strain of NDV and paclitaxel significantly enhanced the later anticancer efficacy. The non-tumor-bearing rats injected with the LaSota strain of NDV remained healthy.

**Conclusion:** Herein it is concluded that NDV efficiently and selectively replicates in and kills tumor cells, but not normal cells thus it could be offered as an unprecedented curative strategy to eradicate cancer

### **E-37: INVESTIGATING TOLL-LIKE RECEPTOR AGONISTS AS AN IMMUNOTHERAPEUTIC FOR METASTATIC OSTEOSARCOMA**

Kathryn Wycislo, Holly Pondenis, Bahaa Fadel-Alla, Timothy Fan

**Background:** Osteosarcoma is an aggressive neoplasm with a propensity for lung metastasis. Despite oncologic advances, survival times for osteosarcoma remain stagnant, driving the need for new therapies. Toll-like receptors, a critical component of innate immunity, have potential as anti-cancer immunotherapeutics. Therefore, we sought to determine if toll-like receptor immunotherapy could be useful in metastatic osteosarcoma.

**Objective:** We previously identified the toll-like receptor agonists Pam3CSK4, Poly(I:C), and CpG ODN as candidate agonists for osteosarcoma immunotherapy. Our objective was to determine which candidate agonist would be best suited for evaluation in canine osteosarcoma.

**Methods:** The candidate agonists were employed within a preclinical model of murine metastatic osteosarcoma. Pulmonary tumor burden, splenocyte effector cell populations, and survival time were evaluated to determine the optimal toll-like receptor agonist. This optimal agonist was then evaluated in a small number of dogs with spontaneous osteosarcoma.

**Results:** Poly(I:C) and CpG ODN immunotherapy exhibited gross and histologic reductions in osteosarcoma pulmonary metastatic burden. CpG ODN therapy resulted in altered splenocyte effector cell populations and clinically-relevant increases in survival time. Pam3CSK4 did not reduce tumor burden or increase survival. When evaluated in pet dogs with osteosarcoma, a single injection of CpG ODN could produce measurable immune responses.

**Conclusions:** CpG ODN is effective in reducing metastatic burden and prolonging survival in a preclinical murine model of metastatic osteosarcoma. CpG ODN also incites measurable immune responses in dogs with osteosarcoma. These findings support further investigation into CpG ODN's mechanism of action and its potential use as an osteosarcoma immunotherapeutic.

## **E-38: EXPLORING THE POTENTIAL LINK BETWEEN REGENERATIVE CAPACITY AND CANCER INITIATION IN ZEBRAFISH**

Vassili Kouprianov, Heather Shive

**Background:** Adult zebrafish have robust stem and progenitor cell populations in the optic nerve pathway (ONP), enabling optic nerve regeneration after optic nerve injury (ONI). We have observed that the ONP is a cancer-prone site in zebrafish with mutations in *brca2* and *tp53* (*brca2*-mutant/*tp53*-mutant), leading us to examine the relationship between regenerative potential and cancer predisposition in this model.

**Methods:** We evaluated the short-term and long-term effects of surgically induced unilateral ONI in zebrafish from three genotypic groups (*brca2*-mutant/*tp53*-mutant, *tp53*-mutant, and wild-type).

**Results:** In short-term studies, we observed significantly increased cellularity in the injured optic nerve in *brca2*-mutant/*tp53*-mutant at both three days and two weeks post-injury. Evaluation of markers for stem and progenitor populations in the ONP (*blbp*, *sox2*, and *sox10*) demonstrated a sustained increase in *blbp* expression, a marker for radial glial cells, in the injured optic nerve from *brca2*-mutant/*tp53*-mutant zebrafish that was not observed in *tp53*-mutant or wild-type cohorts. In long-term studies, there was no overall change in the prevalence of ocular tumor development in *brca2*-mutant/*tp53*-mutant or *tp53*-mutant zebrafish following ONI compared to a control cohort. An increased prevalence of ocular tumors on the injured side was observed in *brca2*-

mutant/*tp53*-mutant zebrafish, but not *tp53*-mutant zebrafish. No ocular tumors were observed in wild-type zebrafish following ONI.

**Conclusions:** These findings suggest that the acute injury response to ONI is amplified in *brca2*-mutant/*tp53*-mutant zebrafish, but this response does not significantly influence cancer onset; thus, other factors are likely to drive cancer predisposition in the ONP in *brca2*-mutant/*tp53*-mutant zebrafish.

### **E-39: NOVEL DRUG COMBINATIONS AGAINST DOXORUBICIN-RESISTANT MULTIPLE MYELOMA**

Tyler Peat, Snehal Gaikwad, Beverly Mock

In human multiple myeloma (MM), MYC is activated in 67% of cases and contributes to malignant progression. Despite the many treatments for MM, relapse and drug resistance are commonplace. Combining targeted agents may circumvent MM resistance, reduce doses, and improve patient outcome. We utilized an NCATS high-throughput screening platform of ~1900 small molecules in 47 human MM cell lines to discover new targeted agent combinations for MM. *In silico* correlation analyses revealed 43 candidate drug combinations predicted to be cooperative in inhibiting cell growth. Candidate combinations were evaluated for cooperative targeting of MYC protein expression in L363 MM cells. Ten combinations reduced MYC expression relative to single agent and/or control. Combinations of a topoisomerase-2A (TOP2A) inhibitor and either an aurora kinase A (AURKA), or a Heat Shock Protein 90 (HSP90) inhibitor were particularly synergistic. The effects of select candidate combinations on decreasing survival of L363 MM cells, and an MM cell line selected for resistance to doxorubicin, were then evaluated. L363, RPMI-8226-Dox40 (doxorubicin-resistant), and RPMI-8226-P (parental) cells treated at escalated doses of either drug singly or in combination for 48 hours were assessed via MTS cell proliferation analysis. Cooperative reduction in MM cell viability was observed upon combined TOP2Ai/AURKAi or TOP2Ai/HSP90i treatment in L363 cells, as well as the doxorubicin-resistant MM cell line (RPMI-822-Dox40) and respective parental cell line. These data identify new drug combinations for treating relapsed/resistant MM which may reveal mechanisms of combined drug sensitivity in multiple myeloma. Further testing in animal models will follow.

### **E-40: ESTABLISHMENT AND CHARACTERIZATION OF A NEW CELL LINE FROM CANINE CUTANEOUS LANGERHANS CELL HISTIOCYTOSIS**

Nguyen Son, James Chambers, Thongtharb Antigan, Takuya Kishimoto, Kazuyuki Uchida, Hiroyuki Nakayama

**Background:** Canine cutaneous Langerhans cell histiocytosis (LCH) is a histiocytic proliferative disorder characterized by Langerhans cell proliferation. Canine LCH shares histological and immunohistochemical characteristics with canine cutaneous histiocytoma, though clinical features of LCH such as multiple distribution of the lesions are different.

**Objective:** The aim of this study was to establish a cell line derived from a LCH case and to characterize its phenotype and tumorigenicity.

**Methods:** Autopsy from 5-year-old female French Bulldog LCH case revealed multiple subcutaneous masses with multi-visceral metastatic involvement. Microscopic examination revealed tumor foci in the subcutaneous masses, lymph nodes, spleen, pancreas, kidney and urinary bladder. A cell line (FB-LCH01), obtained from the cutaneous mass was established and examined by immunocytochemistry and Western blotting. Nine female SCID mice were subcutaneously inoculated with FB-LCH01 cells to examine the tumorigenicity.

**Results:** Original tumor cells as well as FB-LCH01 cells were immunopositive for HLA-DR, Iba-1 and E-cadherin, and immunonegative for cytokeratin, CD3, CD20, CD11b, MAC387, CD163 and CD204. All SCID mice developed tumor masses at the injection site. Eight mice also developed metastatic lesions in the lymph nodes, skin, lung, stomach, heart, pancreas, kidney and bone marrow. The xenotransplanted tumor cells maintained the immunohistochemical features of the original canine tumor. By Western blotting, E-cadherin was detected in FB-LCH01 cells, but not in two canine histiocytic sarcoma cell lines (PWC-HS01 and FCR-HS02).

**Conclusion:** The established cell line reflects the nature of canine LCH and can be used as new tool for investigating the patho-tumorigenesis and therapy of the disease.

## Diagnostic Pathology Focused Scientific Session I

November 4, 2018 | 1:30 PM – 5:00 PM

Session Chair: Eric R. Burrough, DVM, PhD, DACVP, Iowa State University, Ames, IA

November 4, 2018

1:35 PM – 1:45 PM

### DEVELOPMENT AND USE OF A DATABASE OF RETROSPECTIVE DIAGNOSTIC DATA OF PSITTACINE BIRDS

Daniel Gibson, Nicole Nemeth, Hugues Beaufrère, Csaba Varga, Leonardo Susta

**Background:** Psittacines birds (e.g., parrots, macaws) are popular household pets, which are also kept in zoos and private collections. Existing veterinary medical literature regarding diagnostic pathology of captive psittacines is based largely on data derived from case reports with an overall lack of systematic approaches.

**Objective:** 1) To create a database summarizing pathological data of psittacine birds submitted to the Ontario Veterinary College and Animal Health Laboratory from 1989-2017. 2) Use the database to assess disease prevalence among taxonomic, and demographic groups, and to review presentation of specific diseases.

**Methods:** The database included, for each case, taxonomic and demographic data, and final diagnoses, which were further categorized by aetiology and severity (i.e., primary, non-primary). Causes of morbidity and mortality were summarized, and statistical analyses were performed to assess for correlations and associations with taxonomic and age groups.

**Results:** Data were retrieved from 1850 birds, representing 46 genera, including most commonly: cockatiels (12%), African gray parrots (12%), and budgerigars (12%). Viral (21%), bacterial (12%), degenerative (11%), metabolic (10%), hemodynamic (8%), and neoplastic (7%) diseases were the most common primary diagnoses. Significant correlations were found between etiologies and demographic groups and further analysis revealed significant associations. For example, viral infections correlated with the genera: *Psittacus*, *Poicephalus*, *Aratinga*, *Pionites*, and *Psittacara*, and were also associated with young age groups considering all, diagnoses genera and age groups.

**Conclusions:** This study is one of few summaries of pathological findings in a large cohort of psittacines, and improves our understanding of psittacine diseases and associated presentations.

November 4, 2018

1:45 PM – 1:55 PM

### SYNOVIAL AMYLOIDOSIS IN THREE ADULT CAPTIVE TIGERS

Olufemi Fasina, Francis Sebastian, Meeja Sula, Linden Craig

We report a case series of three adult tigers: two females and a male (median age 19.7 years). The tigers were euthanized for clinical disease ranging from hindlimb paraparesis to severe respiratory distress. On necropsy, in all cases, the

scapulohumeral and stifle joint synovium and joint capsule were thickened and orange-brown with articular erosions and osteophytes. Thoracic and cervical intervertebral disc degeneration and herniation was present in two cases. Microscopically, the synovial membrane was hyperplastic with papillary projections and infiltrates of lymphocytes, macrophages and hemosiderin-laden macrophages. Within the subintimal stroma and expanding the synovial membrane and tunica media of subintimal arteries were variably sized extracellular aggregates of homogenous, amorphous, basophilic material. The homogeneous material was Congo red positive, potassium permanganate resistant, and displayed yellow and green birefringence under polarized light. The material was also immunohistochemically positive for transthyretin. In one case, amyloid deposition was severe and systemic with amyloid deposition in heart, esophagus, kidney, pancreas, and lungs. Transthyretin is an amyloidogenic protein that normally functions as a retinol and thyroxine transporter; it is produced in the liver, choroid plexus, and retina. Transthyretin amyloidosis is associated with senile systemic amyloidosis and carpal tunnel syndrome in humans. Transthyretin synovial amyloidosis is an uncommon arthropathy in captive felids.

November 4, 2018

1:55 PM – 2:05 PM

## **HISTOLOGIC CHARACTERIZATION OF EMERGENT INFECTIOUS DISEASES WITHIN THE ORNAMENTAL FISH INDUSTRY**

Abigail Armwood, Alvin Camus

International trade in ornamental fish, both capture and culture fisheries, is a multibillion dollar industry, with the United States and European Union representing the world's two largest import markets. Although import/export regulations have increased at various international, national, and state levels, health surveillance and biosecurity measures are often limited. Lack of disease monitoring coupled with the inherently stressful nature of international shipment favor the development and dissemination of infectious disease. This report characterizes disease outbreaks diagnosed since 2010 by the University of Georgia's Aquatic Pathology Service, involving four pathogens emerging in the ornamental fish trade. The agents include *Francisella noatunensis* subsp. *orientalis*, *spaC*-type *Erysipelothrix* sp., megalocytivirus, and betanodavirus. Franscisellosis was diagnosed in four groups of damselfish or fairy wrasse species. Characterized by disseminated granulomas and granulomatous inflammation, lesions are typified by macrophages containing the small, gram-negative coccobacilli in intracytoplasmic vacuoles. An *Erysipelothrix* sp., distinct from *Erysipelothrix rhusiopathiae*, was isolated in diseased tetra species with necrotizing dermatitis and myositis. Acute lesions contained massive numbers of gram-positive bacterial rods that have distinct tropism for connective tissues. Six cases involving fresh, brackish and saltwater fish species had histologic features of megalocytivirus, family *Iridoviridae*, infection characterized by cytomegalic inclusion bodies in multiple organs. Betanodavirus infections caused two outbreaks in species of anthias fish. Histologically, vacuolar degeneration was present in retinal lesions and brains. The ongoing emergence of pathogens in the ornamental fish trade emphasizes the need for routine surveillance and pathologists trained to recognize their diagnostic features, as well as pursue appropriate confirmatory testing.

November 4, 2018  
2:05 PM – 2:15 PM

### **GLOMERULAR ULTRASTRUCTURAL FINDINGS IN PIGS WITH PORCINE DERMATOPATHY AND NEPHROPATHY SYNDROME**

Christopher Siepker, Carrie Schmidt, Cathy Brown

**Background:** Porcine circovirus is a small DNA virus which is most commonly associated with postweaning multisystemic wasting syndrome (PMWS) in piglets. Porcine circovirus type 2 (PCV2) infection in commercial swine is a complex viral infection which most commonly results in severe lymphoid depletion, clinically manifesting as poor body condition and enlarged lymph nodes, causing economic loss worldwide. PCV2 infection has also been associated with Porcine dermatopathy and nephropathy syndrome (PDNS), manifested grossly as dark red to purple discolorations on the skin and kidneys. The light microscopic findings of PDNS are those of an exudative glomerular disease, with erythrocytes, fibrin, and inflammatory cells within Bowman's space. While this unique manifestation of porcine circovirus associated disease (PCVAD) is thought to represent immune-mediated sequela to viral infection, the ultrastructural glomerular lesions have not been previously described.

**Objective:** Evaluate glomeruli in swine with PDNS by ultrastructure for the deposition of immune complexes.

**Methods:** Swine tissues were evaluated by immunohistochemistry (IHC) to confirm circovirus infection and transmission electron microscopy (TEM) was utilized to evaluate the affected glomeruli ultrastructurally.

**Results:** Glomeruli exhibited podocyte effacement and Bowman's space contained abundant fibrin, neutrophils, and erythrocytes. Focal rupture of the glomerular basement membrane was present. Rare mesangial electron dense deposits in occasional glomeruli were consistent with nonspecific trapping.

**Conclusions:** Ultrastructural findings differ from those seen with primary endothelial injury (thrombotic microangiopathy) or immune-complex glomerulonephritis (membranoproliferative glomerulonephritis). The lesions are similar to those described in people with pauci-immune crescentic glomerulonephritis, an Antineutrophil Cytoplasmic Antibody (ANCA)-associated renal vasculitis.

November 4, 2018  
2:15 PM – 2:25 PM

### **A SERIES OF HETEROGENEOUS LYMPHOPROLIFERATIVE DISEASES CO-EXPRESSING CD3 AND MUM-1 IN DOGS AND CATS**

Kelly Hughes, Emily Rout, Paul Avery, A Moore, Anne Avery

**Background:** In humans, both CD3-expressing plasma cell tumors and MUM1-expressing subtypes of peripheral T-cell lymphoma have been described. CD3+ and MUM-1+ plasmacytomas are also rarely reported in the dog.

**Objective:** Describe the variability of lymphoproliferative diseases co-expressing CD3 and MUM-1 in dogs and cats and determine the lineage of these tumors using ancillary diagnostics.

**Methods:** 5 feline and 5 canine tumors co-expressing CD3 and MUM-1 previously identified by immunocytochemistry and/or immunohistochemistry were examined. Ancillary diagnostics included serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE), PCR for antigen receptor rearrangements (PARR), and flow cytometry.

**Results:** Half the cases were anemic and 2 had concurrent thrombocytopenia. 50% of cases had hepatic and splenic involvement and 50% involved the dermis. Additionally, 3 cases were nodal, 1 case involved bone, and 1 feline case was subcutaneous and localized to the tarsus. Cytomorphology varied from well-differentiated plasma cells to poorly-differentiated discrete cells. Among 9 cases with PARR performed, 3 cases had clonal T-cell receptor genes, 4 had clonal immunoglobulin genes and 2 cases were polyclonal. SPE/IFE identified clonal IgA gammopathy in 2 of 3 cases. Flow cytometry results were variable and diagnostic for lineage in 1 of 3 cases. In 2 cases where CD18 expression was examined by IHC, 1 was positive.

**Conclusions:** Both plasma cell and T cell-lymphoproliferative disease in dogs and cats can demonstrate co-expression of CD3 and MUM-1. PARR can be useful to determine lineage. These cases highlight the utility of multiple diagnostic modalities to aid in the comprehensive identification of lymphoma subtypes.

November 4, 2018

2:25 PM – 2:35 PM

#### **MICROTUBULE-ASSOCIATED PROTEIN 2 EXPRESSION IN CANINE GLIOMA**

Elena Demeter, Chad Frank, Daniel Rissi, Andrew Miller

**Background:** In the dog, gliomas represent approximately 40% of all primary central nervous system neoplasms with oligodendrogliomas being the most common. Diagnosis of gliomas often relies on immunohistochemical (e.g. Olig2, GFAP) and molecular studies that complement traditional histologic analysis. In human neuropathology, microtubule-associated protein 2 (MAP2) has emerged as a valuable tool in the diagnosis of gliomas, particularly differentiating low-grade gliomas from other primary neuroepithelial tumors. In canine gliomas, MAP2 expression is currently unexplored.

**Objective:** Determine MAP2 expression in canine oligodendroglioma and astrocytoma.

**Methods:** A total of 52 cases of canine gliomas were studied (oligodendroglioma (33/52), astrocytoma (8/52), and undefined glioma (11/52)). In addition to histologic analysis, Olig2 staining was performed in all cases to confirm the diagnosis of glioma. MAP2 immunohistochemistry was evaluated in all cases. The percentage of positive cells in addition to the staining pattern was determined for all cases.

**Results:** MAP2 immunoreactivity patterns were as follows: less than 25% (13/52), 25-50% (10/52), 50-75% (10/52), and 75-100% (19/52). The staining pattern was a combination of cytoplasmic and perinuclear for all cases of oligodendroglioma and undefined glioma whereas all astrocytoma cases had robust immunoreactivity highlighting cytoplasmic processes.

**Conclusions:** MAP2 is strongly expressed in canine glioma and the pattern of expression suggests it is a useful additional marker to confirm the histologic identity of a glioma. Additionally, MAP2 expression appears to have different staining patterns in oligodendroglioma compared to astrocytoma, as reported in human glioma.

November 4, 2018  
2:35 PM – 2:45 PM

### **SQUAMOUS CELL CARCINOMA WITH CLEAR CELL DIFFERENTIATION IN AN EQUINE EYELID**

Leah Stein, Dodd Sledge, Rebecca Smedley, Matti Kiupel, Tuddow Thaiwong

A 15-year-old Miniature horse mare had a six-month history of an ulcerated mass on the right lower eyelid. An incisional biopsy and a subsequent excisional biopsy were submitted to the Michigan State University Veterinary Diagnostic Laboratory for microscopic evaluation. Histologically, the incisional biopsy was composed of sheets of large neoplastic vacuolated polygonal cells. Few regions contained poorly differentiated neoplastic round to basaloid cells that rimmed the sheets of highly vacuolated polygonal cells. Both vacuolated and basaloid cells exhibited strong perimembranous immunoreactivity for E-cadherin. Vacuolated polygonal cells were histochemically negative for periodic acid-Schiff, mucicarmine, and oil red O, consistent with a diagnosis of poorly differentiated carcinoma. Within the excisional biopsy specimen, there were anastomosing cords and nests of neoplastic squamous epithelial cells along the periphery of the mass. The neoplastic squamous epithelial cells surrounded central vacuolated cells. These findings are consistent with a squamous cell carcinoma with clear cell differentiation. In addition, in the adjacent dermis, there was solar elastosis suggestive of UV damage. A clear cell variant of squamous cell carcinoma is a rare entity in humans that has not been previously described in animals and is often associated with chronic UV exposure.

November 4, 2018  
2:45 PM – 2:55 PM

### **SCHWANNOSIS IN THREE FOALS AND A CALF**

Ileana Miranda, Kyle Taylor, William Castleman, Brian Summers, Andrew Miller

**Background:** Conventionally, oligodendrocytes and Schwann cells populate the CNS and PNS respectively. Ectopic intramedullary nests of Schwann cells are found in human Schwannosis, historically associated with neurofibromatosis type 2, but also recognized following some traumatic spinal cord injuries.

**Objective:** To investigate four suspected cases of Schwannosis in domestic animals.

**Methods:** We studied three foals and one calf, 5- to 11-weeks-old, with progressive neurological signs from birth. Histologic examination and immunohistochemistry, including the Schwann cell markers protein zero (P0) and periaxin, were used.

**Results:** In all animals, at multiple levels of the spinal cord, the primary histologic feature was bilateral plaques of proliferative spindle cells, which predominantly affected peripheral white matter adjacent to dorsal and ventral nerve roots, multifocally surrounded blood vessels, and variably extending to the gray matter. In two cases, haphazardly oriented neural tissue also formed a mass effect that compressed the spinal cord leading to syringohydromyelia. The proliferating cells had strong intracytoplasmic immunoreactivity for P0 and periaxin, and both highlighted the formation of PNS myelin within the spinal cord.

**Conclusions:** Lesion immunoreactivity, morphology, and distribution –proximity to CNS-PNS junctions – closely resembles human Schwannosis. Neonatal presentation suggests a congenital malformation with spontaneous invasion of the CNS by PNS-derived Schwann cells.

November 4, 2018

2:55 PM — 3:00 PM

Veterinary Student Oral Presentation

### **OPTIMIZATION OF METHODS TO STUDY THE EFFECT OF CANNABINOIDS ON IMMUNE RESPONSES IN STIMULATED CANINE PBMCs**

Clare Brown, Evangel Kummari, Todd Archer, Barbara Kaplan

Current canine autoimmune disease treatments are not ideal due to limited efficacy or adverse side effects. Cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), are compounds from the marijuana plant (*Cannabis spp.*) that have gained attention as potential treatments for autoimmune diseases. In mice it has been established that they are immunosuppressive and provide benefit in autoimmune models, but in most other species there exists little data. The aim of this project was to examine the potential of cannabinoids as effective treatments for canine autoimmune diseases by determining whether they are immunosuppressive in canine peripheral blood mononuclear cells (PBMCs) in vitro. PBMCs were isolated from canine blood and treated with CBD or THC, followed by stimulation with ConA, LPS, or PMA/ionomycin (PI). Carboxyfluorescein succinimidyl ester (CFSE) staining was used in conjunction with flow cytometry to assess lymphocyte proliferation. RNA was also isolated to determine gene expression of cytokines interleukin-2 (IL-2) and interferon gamma (IFN-gamma) via qPCR. After assessing PBMC responsiveness under various conditions, the studies focused on PI as it was the most effective stimulant. Results from both flow cytometry and qPCR indicated that CBD and THC could affect proliferation and cytokine production, but that it is dog-specific; some dogs exhibited no effect, while others exhibited modest suppression by CBD and THC. IFN-gamma consistently exhibited a higher magnitude of expression change than IL-2. These findings provide important preliminary insight into the potential of these compounds for canine autoimmune diseases and establish a knowledge base of effective methods for future studies.

November 4, 2018  
3:30 PM – 3:35 PM

### **STANDARDIZATION OF MITOTIC COUNT IN TUMORS**

Donald Meuten, Fran Moore, Margaret Miller, Paul Stromberg, Joshua Webster, John Munday, Derick Whitley

Counting mitotic figures is one of the oldest and most widely used methods to estimate cell proliferation and provide a prognosis for tumors. Studies in veterinary oncology incorrectly report mitotic index (MI) in place of mitotic count (MC) and state 10 high power fields (HPF) as the area in which mitotic figures are enumerated. However, 10 HPFs is not a standard unit. Depending on the ocular and the objective, the area in 10 HPF varies 30-600%. For MC to be a meaningful parameter in tumor prognosis, the area in which mitoses are counted must be defined and standardized.

The diameter of a field of view (FOV) is calculated from the ocular field number (FN) and the magnification of the objective. Today, the standard ocular FN is 22, so  $22 \text{ mm}/40\times = 0.55 \text{ mm}$  diameter. The area of a circular HPF =  $0.237 \text{ mm}^2$ ;  $10 \text{ HPF} = 2.37 \text{ mm}^2$ . The MC in 10 HPF is reported as number mitotic figures/ $2.37 \text{ mm}^2$ . For whole slide digital images, the area of the field depends on the magnification and size of the display on the monitor. A micrometer or software measuring tool is used to calculate FOV area in the rectangular image: length X width = FOV  $\text{mm}^2$ . To determine the number of fields needed for the MC, divide  $2.37 \text{ mm}^2$  by the digital image FOV area.

Standardization of area in which mitoses are counted will help ensure MC uniformity among pathologists using glass slides, scanned images, or automated systems.

November 5, 2018  
3:35 PM – 3:40 PM

### **THYMOMA IN A COMMON BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS) RESEMBLING HUMAN TYPE B3 AND STAGE II THYMOMA**

Wen-Ta Li, Chieh Lo, Chian-Ren Jeng

**Background:** Thymomas are neoplasms derived from thymic epithelial cells with benign lymphoid proliferation. Although thymomas have been reported in a variety of wildlife species, no cases of thymoma have been reported in either captive or free-ranging cetaceans.

**Case presentation:** An adult female bottlenose dolphin was stranded and found dead on the east coast of Taiwan. Necropsy and tissue sample collection for histopathology were performed.

**Results:** There were several round-shaped wounds (caused by cookiecutter sharks) on the left-dorsal aspect of body trunk. A fixed, well-encapsulated, smooth bosselated,  $5 \times 4 \times 3 \text{ cm}$  mass was found in the middle mediastinal region, and the cut surface showed variable-sized, jigsaw puzzle-like, white to tan nodules demarcated by white sclerotic septa. The mass comprised lobules of polygonal neoplastic cells associated with

scattering aggregates of CD3+ T-lymphocytes and separated by thick fibrotic stroma. Aggregates of neoplastic cells were noted in the capsular area. The neoplastic cells were intensely positive for cytokeratin and P63, focally positive for CK19, and negative for CD5, CD117, vimentin, thyroid transcription factor-1, synaptophysin and chromogranin A.

**Conclusion:** In humans, thymomas are classified into five subtypes (A, AB, B1, B2 and B3) and four stages (I to IV) by the World Health Organization histologic classification and Masaoka-Koga staging system, respectively. Patients with type B3 or stage IV thymoma usually have a relatively worse prognosis. Based on the anatomic location and the histopathologic/immunohistochemical characteristics, the neoplasm was considered a thymoma resembling human type B3 and stage II thymoma.

November 4, 2018

3:40 PM – 3:45 PM

### **HYDROCEPHALUS AND NEONATAL MORTALITY ASSOCIATED TO COMBINED DEFICIENCY OF VITAMIN A AND FOLIC ACID (VIT B9) IN MATERNAL RABBITS**

Abelardo Morales, Manuel Moya-Acosta, Carmen Esteves, Yonelsi Brito, Emilio Suniaga

**Background:** The aim of this study was to describe hydrocephalus and neonatal mortality associated with combined deficiency of vitamin A and folic acid (vit B9) in maternal rabbits. An increase in neonatal mortality (45%) was observed in the production of rabbits. Some kits presented with hydrocephalus, cranioschisis, deformations of the limbs, blindness, and ataxia.

**Results:** At necropsy, the internal lamina of the frontal sinus was not fused with the occipital bone leading to bone defects in both sides and communication of the brain with the external lamina of the frontal sinus as well as with the temporal and occipital bones. This deformity provided a remarkable space around the brain, allowing cerebrospinal fluid (CSF) to leak into the sinus space through the defect. In addition, two large fluid-filled cystic cavities bounded by a thin rim of brain tissue were found in the cerebral hemisphere. The enlarged lateral ventricles contained an abnormally large volume of CSF forming hydrocephalus. Histologically, only hemodynamic changes and compression atrophy affecting the brain and cerebellum were observed. Other findings included metaplasia of renal proximal convoluted tubules and lymphoid depletion.

**Conclusion:** From these findings, hydrocephalus and neonatal mortality was associated with combined deficiency of vitamin A and folic acid (vit B9) detected in maternal rabbits. A bromatological analysis of the feed is in underway.

November 4, 2018

3:45 PM – 3:50 PM

**CANINE CUTANEOUS AND SUBCUTANEOUS SOFT TISSUE SARCOMAS:  
IDENTIFICATION OF THERAPEUTIC TARGETS THROUGH GENE EXPRESSION  
LARGE-SCALE STUDY**

Renee Laufer-Amorim, Juliano Nobrega, Sandra Linde, Lissandro Conceição, Rafael Torres-Neto, Fabricia Loures

**Background:** Peripheral nerve sheath tumors (PNSTs) are soft tissue sarcomas that often affect dogs. The treatment of choice is surgical, however, new therapeutic modalities, such as the target molecular therapy, would benefit patients with the impossibility of complete surgical resection.

**Objective:** The aim of this study was to evaluate global gene expression profile of canine subcutaneous PNSTs compared to normal subcutaneous tissues (N) in order to identify altered genes in tumors with potential to be used as therapeutic targets.

**Methods:** RNAs from 30 PNSTs and three N samples were evaluated using the GeneChip® 1.0 ST Array platform. The candidate genes were validated by RT-qPCR in the same array samples and in independent group of cases (n = 22).

**Results:** A total of 2458 differentially expressed genes in PNSTs compared to the N group were found. Six genes (*CDK4*, *COL3A1*, *EPHA3*, *MMP2*, *PLK4* and *PPARG*) were selected based on their function, fold change and p values, as well as for being drug targets. *CDK4* (P = 0.0001), *COL3A1* (P = 0.0010), *EPHA3* (P = 0.0002), *MMP2* (P = 0.0023) and *PLK4* (P = 0.0003), all results confirmed by gene expression.

**Conclusions:** The global gene expression profile of PNSTs revealed genes with potential for molecular targeted therapy for these tumors, which may particularly benefit patients with unresectable tumors or with narrow surgical margin.

Financial support: CNPq

November 4, 2018

3:50 PM – 3:55 PM

Veterinary Student Presentation

**PLATELET-ENDOTHELIAL ASSOCIATIONS MAY IMPACT THE PATHOGENESIS  
OF CYTOMEGALOVIRUS INFECTION IN MICE**

Brenna Daly, Alicia Braxton, Griffin Cyphers, Shefali Vijay, Jacqueline Brockhurst, Alyssa Chalmin, Kevin Najarro, Karl Johnson, Catherine Cryer, Selena Guerrero-Martin, Rupkatha Mukhopadhyay, Yu-Pin Su, Ravit Boger, Kelly Metcalf Pate

Platelet decline is a hallmark of many acute viral infections, including cytomegalovirus (CMV) infection in humans and mice. Specific mechanisms of platelet decline, such as platelet sequestration in interactions with other cells, can directly impact viral pathogenesis through receptor- and cytokine-mediated signaling. The immunopathogenesis of platelet decline in CMV infection is incompletely understood. Preliminary data show that experimental platelet depletion in CMV-infected mice

prevents CMV replication in salivary glands that occurs in non-depleted mice 21 days post-infection. It has also been demonstrated that platelet sequestration in monocyte, lymphocyte, or neutrophil aggregates does not cause platelet decline in CMV infection. We hypothesized that platelet-endothelial associations (PEAs) contribute to platelet decline during acute CMV infection in mice and that PEAs, if present, form when circulating platelets are required for CMV replication in salivary glands. Forty-two juvenile male BALB/c mice infected with  $3 \times 10^6$  plaque-forming units of CMV were euthanized on days 0, 3, 8, or 21 for tissue collection. To detect PEAs in salivary glands, immunohistochemistry was used to label platelets with anti-CD41 antibody and a hematoxylin counterstain was used to visualize tissue architecture. The percentage of PEA-positive vessels increased following CMV infection and was statistically significant 21 days post-infection compared to uninfected controls ( $P=0.0125$ ). This suggests that PEA formation contributes to platelet decline and occurs concurrently with viral replication in salivary glands. Future work will assess whether PEAs are required for CMV replication and determine if inhibiting PEA formation could decrease CMV replication in infected patients.

November 4, 2018

4:00 PM – 4:05 PM

#### **INTRAOCULAR NEURAL HETEROTOPIA IN A MOROCCAN UROMASTYX**

Marta Mainenti, Chad Clancy, Arnaud Van Wettere

**Background:** Neural heterotopia is a rare developmental anomaly consisting of neural tissue in aberrant locations. In humans, heterotopic neural tissue most frequently forms masses in the scalp, nose, lip, nasopharynx, oropharynx, tongue, ear, and orbit. In animals, neural heterotopia has been reported twice in the skin of the frontal head and pharynx of kittens.

**Objectives:** To report a case of intraocular neural heterotopia in a Moroccan Uromastyx.

**Methods:** A 14.3 g, juvenile, female, Moroccan Uromastyx (*Uromastyx acanthinurus nigriventris*) that died unexpectedly after brief rehoming was necropsied.

**Results:** Necropsy revealed minimal intra-coelomic fat stores and small numbers of intestinal nematodes. Eye cross sections revealed one, 4 x 5 mm, grey to white, well-demarcated, expansile, soft mass in the vitreous chamber in each eye. Histologically, both intraocular masses were composed of neuropil with rare glial cells and capillaries, and were contiguous with the optic nerve. No mitotic activity, necrosis, atypia, or increased vascularity were identified. This uromastyx also had metastatic skeletal mineralization.

**Conclusions:** Given the neuropil present in the eyes was well differentiated, a diagnosis of intraocular neural heterotopia was made. Death was attributed to a combination of emaciation and potential vitamin D toxicity. Vision impairment due to the space-occupying, intraocular collection of neuropil is suspected but could not be

clinically verified. To the authors' knowledge, this is the first case of intraocular neural heterotopia reported in veterinary medicine.

November 4, 2018

4:05 PM – 4:10 PM

### **CILIATE INFECTION ASSOCIATED WITH CUTANEOUS ULCERATION IN SEA PENS (ORDER: PENNATULACEA)**

Emily Corbin, Michael Garner, Elise LaDouceur

**Background:** Protozoal diseases are uncommonly described in animals from the class anthozoa, which includes corals, anemones, and sea pens. This study describes disease presentations and lesions associated with ciliate infections in sea pens (order: pennatulacea). Although recently associated with significant disease in coral, disease associated with ciliate infection has not been previously described in sea pens.

**Methods:** Archives of Northwest ZooPath were searched for sea pens with ciliate infections. Histology was reviewed, and scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed.

**Results:** Four sea pens were identified with ciliate infections. Clinical observations included dull coloration, cutaneous erosion, cutaneous sloughs, and lateral recumbency. In each case, ciliates were seen in cytologic examination of skin scrapings. One animal was euthanized, two were found dead, and one was biopsied and subsequently lost to follow up. Histologically, ciliate infection was consistently associated with cutaneous ulceration, necrosis, and infiltration of amoebocytes. Ultrastructurally, SEM of protozoa revealed dense cilia distributed over the entire body. TEM revealed anatomic detail of the cilia, alveoli, kinetome, (i.e. organelle system associated with cilia), and macronucleus.

**Conclusions:** As ciliates were consistently associated with ulcers in this series, it is suspected that this infection is a substantial contributor to cutaneous ulceration. It is unclear if the ciliates are the cause of ulceration and subsequent mortality, or if ciliates secondarily colonize ulcers that are caused by other factors. This is the first description of ciliate-associated disease in sea pens.

November 4, 2018

4:10 PM – 4:15 PM

### **PANCREATIC CYSTIC LESIONS IN LABORATORY MICE FROM PREDOMINANTLY NOD AND NOD-DERIVED STRAINS**

Jose Vilches-Moure, Rosalinda Doty, Denise Imai-Leonard

**Background:** Reports of pancreatic cysts in mice are generally associated with polycystic disease, or as a feature of the overall lesions observed in pancreatic cancer models. However, pancreatic cystic lesions in mice outside those scenarios are exceedingly rare.

**Objective:** To investigate and characterize pancreatic cystic lesions in a cohort of 10 purpose-bred laboratory mice.

**Methods:** Animals were submitted for evaluation of abdominal distension and/or a palpable abdominal mass. Five of ten animals were NOD.Cg-Prkdc<sup>scid</sup>IL2rg<sup>tm1Wjl</sup>/SzJ [aka NOD scid gamma, or NSG] mice. Three of ten animals were from the following strains (one of each): NOD/ShiLtJ [NOD], NOD.CB17-Prkdc<sup>scid</sup>/J [NOD scid], and NOD.B-(D3Mit93-D3Mit124)(D4Mit114-D4Mit142)/1112MrkJ [NOD.c3.c4]. Two of ten animals were not NOD-derived strains: BTBR.V(B6)Lep<sup>ob</sup>/WiscJ [BTBR obese] and CBySmn.CB17-Prkdc<sup>scid</sup>/J [BALB scid].

**Results:** Grossly, cystic lesions were thin-walled with prominent surface vessels. Histologically, dilated spaces were surrounded by a thin fibrous capsule often accompanied by early fibroplasia and/or edema (7/10). Cystic spaces were lined by cuboidal (10/10) to columnar (7/10) epithelium. In all cases there was lymphocytic infiltration of the fibrous capsule, and most cases also had neutrophilic (8/10) and histiocytic (9/10) infiltrates. There was occasional hemorrhage (5/10), varying degrees of pancreatic acinar atrophy (6/10), and fibrosis (5/10).

**Conclusions:** Gross or histologic evidence of luminal obstruction was absent, and the driver of these cystic dilations could not be definitively determined. Animals were predominantly, but not exclusively, of NOD and NOD-derived backgrounds. Although regarded as rare, pancreatic cystic lesions may be overrepresented in NOD and NOD-derived strains.

November 4, 2018  
4:15 PM – 4:20 PM

#### **NEUROLOGIC AND OCULAR LESIONS IN 8-DAY-OLD BROILER BREEDERS**

Susan Williams, Karen Grogan, Sarah Tilley

A northeast Georgia poultry company submitted live and dead 8-day-old chicks with neurological signs and ocular lesions to the Poultry Diagnostic and Research Center, University of Georgia, from 3 different houses on the same farm. Mortality started on Day 4 and the first week mortality was higher than normal, ranging from 2.5-3.5%. Birds from 2 houses had opaqueness to one or both eyes. The other house had conjunctivitis. All houses had birds with neurologic signs of head tremors and ataxia. Grossly there were caseous lesions in the brains of affected chicks. Evidence of a bacterial infection included pericarditis, yolk sac infection, and polyserositis in all houses. Bacterial swabs from the brain, yolk sac, eye, bone marrow and heart were submitted for isolation and identification along with sensitivity. Brains and eyes were collected and fixed in 10% formalin for histopathology. There was acute multifocal necrotizing meningoencephalitis with Gram negative rods and severe necrotizing panuveitis with Gram negative rods and cataract formation. Pure cultures of *Pseudomonas aeruginosa* were isolated from the brains, eye, heart, bone marrow and yolk sac from all 3 houses.

November 4, 2018  
4:20 PM -- 4:25 PM  
Veterinary Student Presentation

#### **CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF FELINE DIFFUSE**

## **IRIS MELANOMA**

Maylin Akella, Ian Herring, Andrew Enders, Kayla Waler, Tanya LeRoith

This report describes the clinical presentations and the histopathological findings of feline diffuse iris melanoma (DIM). Of twenty-two feline globes submitted to Virginia Tech Animal Laboratory Services (ViTALS) at Virginia-Maryland College of Veterinary Medicine since 2013, seven were diagnosed histologically as feline DIM. In all cases, the owners elected enucleation due to progressive iris pigmentation, regions of raised iridal pigmentation and/or secondary elevated intraocular pressure. Histologic findings ranged from benign iridal hyperpigmentation to iris melanoma with scleral and choroidal invasion. Feline DIM is a progressive disease with a highly variable rate of progression, and it is difficult to predict the metastatic potential of the neoplasm. Clinically, extended pigmentation to the drainage angle, exfoliated cells in the aqueous humor, glaucoma, and involvement of posterior iris epithelium are commonly used as the indicators of higher risk of metastasis. Histopathologically, high mitotic index, tumor necrosis, extrascleral extension, and choroidal invasion are associated with increased metastatic rate. Here, we compare the clinical presentations and histopathological characteristics in feline DIM.

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4:30 PM – 4:35 PM

## **EVALUATING THE EFFECTS OF VARIOUS DECALCIFICATION PROTOCOLS ON IMMUNOHISTOCHEMICAL STAINING IN ZEBRAFISH (DANIO RERIO)**

Danielle Meritet, Christiane Loehr, Sean Spagnoli, Kay Fischer

**Background:** Both fixation and decalcification can alter protein structure in tissues and may impact the efficacy of primary antibodies routinely used in immunohistochemistry.

**Objective:** We investigated the effect of commonly applied fixation and decalcification protocols on immunohistochemical staining in zebrafish.

**Methods:** Zebrafish were fixed for varying times in Dietrich's fixative or 3.4% neutral buffered formaldehyde followed by decalcification with EDTA. Three independent observers evaluated sections stained for cytokeratins, vimentin, GFAP, S100, chromogranin A, and muscle actin by immunohistochemistry.

**Results:** Overall, interobserver variation was moderately high. The type of fixative had different effects for individual markers, reducing or increasing staining intensity; a negative time-dependent effect was detected for several antibodies. Of note, the use of Dietrich's fixative greatly reduced the staining intensity for the neural markers: GFAP, Chromogranin A and S100. The decalcification protocols yielded unexpected results, with longer durations leading to more intense staining scores for one marker.

**Conclusions:** No single fixation protocol worked best for all antibodies. We do recommend NBF/CalExII for immunohistochemical studies of neural tissue in zebrafish.

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4:35 PM – 4:40 PM

### **RENAL TUBULAR DYSGENESIS WITH CONCURRENT OSTEOGENESIS IMPERFECTA, DENTINOGENESIS IMPERFECTA, AND GROWTH ARREST IN A YOUNG DOG**

William Sills, Roy Pool, Katie Kelly

**Background:** An 11-month old, male Staffordshire Bull Terrier dog died after a long history of growth arrest, joint laxity, recurrent respiratory infections, and recurrent juvenile cellulitis.

**Objective:** To describe a case of concurrent renal tubular dysgenesis (RTD) and skeletal and dental malformations in a young dog.

**Methods:** A full necropsy was conducted, and all major tissues, including several sections of long bones and mandible, were examined histologically.

**Results:** Gross evaluation revealed small stature, low body weight (4.2 kg), and severely shortened long bones of all limbs. The teeth were disproportionately large and soft with dull enamel. The frontal and maxillary sinuses were markedly narrow to absent with deformed nasal turbinates. Long bones exhibited severe cortical osteopenia and widespread depletion of cancellous bone, consistent with osteogenesis imperfecta. Teeth lacked dentin in the pulp cavity (dentinogenesis imperfecta). There was renal tubular dysgenesis (RTD) with nearly diffuse malformation of proximal tubules, characterized by narrow, diminutive hypercellular tubules with narrow to indiscernible lumens and thickened basement membranes which were embedded within abundant fibrous connective tissue and interspersed between mature glomeruli. Other findings included severe pulmonary and widespread vascular mineralization.

**Conclusions:** RTD is a rare human clinical fetal disorder characterized by the absence or poor development of proximal tubules. RTD is rarely reported in veterinary species but this case demonstrates that this rare renal malformation can occur with complex congenital skeletal disease; there is no clear shared association between these renal and skeletal malformations.

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4:40 PM – 4:45 PM

### **ACUTE DISSEMINATED CHAGAS DISEASE IN A PREGNANT RHESUS MACAQUE (MACACA MULATTA)**

Michael Betley, Greg Wilkerson, Stanton Gray, Wallace Baze

The protozoan *Trypanosoma cruzi* causes Chagas disease, a neglected tropical disease that affects millions of people in Latin America. The acute phase of infection commonly results in mild, non-specific clinical signs while the chronic phase causes cardiomyopathy, megaesophagus or megacolon in 30% of infected humans over their lifetime. All mammals are susceptible to *T. cruzi*, and natural and experimental infections serve as models for studying the pathogenesis of the disease. Rhesus

macaques (*M. mulatta*) at the MD Anderson Keeling Center for Comparative Medicine and Research in Texas are sporadically naturally infected with *T. cruzi*. We detail an atypical, severe, acute to subacute, disseminated *T. cruzi* infection in a pregnant rhesus monkey. The fetus died *in utero*, and following a Caesarian section to remove the fetus and placenta, the macaque developed endometritis, subcutaneous hindlimb edema and progressive hindlimb paresis. Histological examination unexpectedly discovered extensive inflammatory lesions with numerous protozoal amastigotes in the spinal cord, cerebral cortex, retinal pigment epithelium, heart, esophagus and many skeletal muscles. Historical *T. cruzi* IgG titers from this animal were negative, although affected tissues collected at necropsy were confirmed to be positive for *T. cruzi* infection through PCR. The ocular lesion identified in this case has not been reported previously in non-human primates and the extensive dissemination with neurotropism is a highly unusual presentation for acute *T. cruzi* infection in monkeys. Adaptive immunity is inhibited during pregnancy, and it is hypothesized that an inhibited adaptive response may have contributed to the extensive disease in this unique case.

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## **SUDDEN UNEXPECTED NATURAL DEATH OF THREE DOGS WITH PULMONARY HEMORRHAGE**

Ikki Mitsui, Yoshio Kawamura

**Background:** Pulmonary hemorrhage (PH) is a rare autopsy finding in animals. Listed causes of PH are hemorrhagic diathesis, septicemia, trauma, hemangiosarcoma, *Leptospira*, to name a few. Diffuse pulmonary hemorrhagic syndrome of human includes Goodpasture syndrome, idiopathic pulmonary hemosiderosis, and polyangitis with granulomatosis, all of which are of established or suspected autoimmune etiology.

**Objective:** The investigation was conducted to clarify the cause of both demise and PH and their relationship in three canine autopsy cases.

**Methods:** Three “sudden death” cases with PH were examined by full autopsy, histopathology, histochemistry (Gram, periodic acid-Schiff, phosphotungstic acid hematoxylin stains), bacterial culture of tissue swab, and broad-range polymerase chain reaction for bacterial and fungal pathogens.

**Results:** A 2-month-and-3-week-old intact male Pomeranian had pulmonary edema and congestion. These and PH were suspected to be caused by septicemia due to bacterial translocation from superficial necrotizing ileitis. A 6-year-and-5-month-old castrated male Toy Poodle showed pulmonary edema and congestion and acute tubular injury (ATI). Subtle myocarditis was suspected to cause acute cardiac failure in this dog. The third case was a 12-year-and-4-month-old spayed female Yorkshire Terrier with severe tracheal collapse, mitral valvular myxomatous degeneration, ATI, glomerular basement membrane thickening, and multifocal pulmonary atelectasis. The third dog’s PH was suspected to be caused by sudden fatal tracheal collapse though Goodpasture-syndrome-like condition could not be completely ruled out.

**Conclusions:** Etiology of canine PH is diverse and its determination requires laborious laboratory work. Causation between PH and other organ's abnormality should be carefully sought for possible discovery of novel animal syndrome.

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4:50 PM – 4:55 PM

## **ULCERATIVE VEGETATIVE AND CAVITATING BACTERIAL AORTIC VALVULAR ENDOCARDITIS IN TWO DOGS**

Tatiane Terumi Negrão Watanabe, Sarah Shippy, Fabio Del Piero

**Background:** Valvular endocarditis is common in farm animals but it is seldom observed in dogs. Cavitating endocarditis has been sporadically associated with *Bartonella* spp.

**Methods:** A 2.5-year-old, intact male, Boxer dog with a sudden death (case1) and a 4-year-old, intact male, American Bulldog with a 4-day history of anorexia, lethargy, vomiting, bloody diarrhea, and weight loss (case 2) were submitted for postmortem examination.

**Results:** In both cases, the aortic semi-lunar valves were irregular with loss of portions of the leaflets and effaced by brownish, irregular, friable, dry exudate. The endocardium behind the valves was ulcerated with significant focal loss of myocardium forming a 3.0 cm oval cavitation extending for the valve transversal length. Similar lesions affected the left atrioventricular valve (case 1). The aortic valve was replaced by neutrophils and macrophages mixed with abundant fibrin, cellular necrotic debris and edema with numerous Gram-positive cocci and Gram-negative rods and filamentous bacteria. The heart and kidney were affected by multiple infarcts and hemorrhage. No bacteria were isolated from valvular samples. PCR for detection of *Bartonella* spp. in paraffin-embedded tissue is pending.

**Conclusions:** Here we describe the findings of an unusual bacterial aortic bicuspid valvular endocarditis with associated necrosis and cavitation of the adjacent myocardium in two dogs.

## **Diagnostic Pathology Focused Scientific Session II**

November 6, 2018 | 1:30 PM – 5:00 PM

Session Chair: Eric R. Burrough, DVM, PhD, DACVP, Iowa State University, Ames, IA

November 6, 2018

1:30 PM – 1:40 PM

### **IDENTIFICATION OF PORCINE ASTROVIRUS TYPE 3 IN CENTRAL NERVOUS SYSTEM TISSUE FROM SWINE WITH NEUROLOGIC DISEASE AND ENCEPHALOMYELITIS: DIAGNOSTIC INVESTIGATION, VIRUS CHARACTERIZATION, AND RETROSPECTIVE ANALYSIS OF HISTORICAL CASES**

Franco Matias Ferreyra, Paulo Arruda, Eric Burrough, Laura Bradner, Qi Chen, Vickie Cooper, Rachel Derscheid, Phillip Gauger, Karen Harmon, Ben Hause, Melissa Hensch, Igor Honorato Gatto, Ganwu Li, Darin Madson, Pablo Piñeyro, Kent Schwartz, Gregory Stevenson, Ying Zheng, Chenghuai Yang, Bailey Arruda

Porcine astroviruses (PoAstVs) are distributed worldwide with five recognized lineages (PoAstV1-5). Recently, astroviruses have been linked to central nervous system (CNS) disease in humans, cattle, sheep, and mink. Over a 9-month period, swine neurologic cases originating from a multisite swine production flow representing a population of 4-12-week-old pigs and adult sows were submitted to the Iowa State University Veterinary Diagnostic Lab (ISU VDL). As common viral and bacterial pathogens were not detected by PCR or isolated, respectively, next-generation sequencing (NGS) was used to identify and genetically characterize a PoAstV3 in CNS tissue of one piglet and three sows with neurologic signs and encephalomyelitis. Metagenomic sequencing from CNS samples identified a near complete genome (6461 nt) of the virus, and genome phylogenetic comparison placed it under the same cluster as another PoAstV3, yielding the highest homology (92.2% nt identity) with PASTV3/ US/MO/123. Notably, the genome sequence of this PoAstV3 shared the highest nt identity to those of previously described neurotropic astroviruses compared to other PoAstVs lineages. Due to a high percentage of swine neurologic cases without a definitive etiology and to better understand the pathophysiology of PoAstV3, a retrospective analysis of swine neurologic cases of unknown etiology and lesions compatible with a viral agent was undertaken. PoAstV3 was detected by RT-qPCR and *in situ* hybridization in CNS tissue from pigs in varying production categories beginning in 2010, the earliest year samples were available. Based on these data, PoAstV3 appears to be a recurring putative cause of viral encephalomyelitis in swine.

November 6, 2018

1:40 PM – 1:50 PM

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

K. Paige Carmichael, Mauricio Seguel, James Stanton

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

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

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

November 6, 2018

1:50 PM – 2:00 PM

#### **OUTBREAK OF MALIGNANT CATARRHAL FEVER IN FIVE FORMOSAN SEROWS (*CAPRINCORNIS SWINHOEI*) IN TAIWAN**

Ji-Hang Yin, Hue-Ying Chiou, Jiunn-Wang Liao

**Background:** Malignant catarrhal fever (MCF) is a viral disease primary in the order Artiodactyla and presents with lymphoproliferation, vasculitis, erosive-ulcerative mucosal lesions. Ovine Herpesvirus 2 (OvHV-2) has been recognized as one of the important groups in Gamma herpesvirinae related with sheep-associated MCF.

**Case Description:** Five out of six, adult, intact female, Formosan serows (*Capricornis swinhoei*) from the same zoo were presented to the Animal Disease Diagnostic Center, National Chung Hsing University with a history of lethargy, slow movement, ataxia, diarrhea, and were found acutely dead within six days. No signs of illness had been observed previously.

**Results:** Grossly, the right and left cranial and middle lung lobes were diffusely mottled pink to dark brown and firm. The hilar lymph nodes were moderately swollen, and had multiple dark red, pinpoint to 0.3 x 0.1 cm smooth areas. The papillary muscles of left ventricle had multiple, irregular, smooth, dark red, 0.2 x 0.4-0.5 x 0.7 cm areas. The mucosa of the abomasum was diffusely red. Histologically, multiple organs, including lung, hilar lymph nodes, liver, heart, urinary bladder, and uterus had invariable extent of vasculitis, predominantly consisting of lymphocytes and plasma cells, in addition to fibrinous thrombi. Polymerase chain reaction and DNA sequencing identified identified OvHV-2 as the etiologic agent in this case.

**Conclusion:** We herein present a case of malignant catarrhal fever attributed to the OvHV-2 infection. Close direct and indirect contact with the asymptomatic reservoir, Barbados Black belly sheep, is considered the most likely route of infection.

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2:00 PM – 2:10 PM

**VETERINARY FORENSIC PATHOLOGY: CASES FROM AN EMERGING DISCIPLINE**

Beverly McEwen

Animals may be victims, evidence or perpetrators of a crime. When discovered at scenes of serious crimes against people, animals are often both victim and evidence. Internationally, submissions of animals to veterinary diagnostic laboratories from law enforcement agencies have dramatically increased over the past 15 years. Reasons for this are speculative but factors likely include changes in legislation and mandatory reporting of suspected animal abuse by veterinarians. Yet, animal abuse rarely occurs in isolation – when animals are abused, people are at risk and when people are abused, animals are at risk. Animals may be sentinels for domestic abuse and cruelty to a pet is a recognized mechanism of psychological control over a partner: no species of animal is immune to these crimes. The link between animal abuse and concurrent or predicted interpersonal violence is well established. Medical examiners, investigators and attorneys, however, may not be aware that veterinary pathologists are employed at universities or diagnostic laboratories to perform postmortems, or that some have developed expertise in the pathology of animal abuse and neglect. Veterinary pathologists in this emerging subspecialty are in the fortunate position of learning from examples of systemic failings in medical forensic pathology, such as inadequate oversight, training and certification. They can also benefit from the recent advances in several jurisdictions to rectify these issues. Cases submitted to the Animal Health Laboratory, University of Guelph, by law enforcement agencies for postmortem will be used to illustrate examples of animal cruelty and the context in which they arose.

November 6, 2018

2:10 PM – 2:20 PM

**COUNTING MITOTIC FIGURES**

Donald Meuten, Fran Moore, Margaret Miller, Harold Tvedten, Joshua Webster, Derick Whitley

Utilization of a standardized approach to assessing a mitotic count (MC) is necessary to compare results among laboratories. Consensus is needed to define the site of the tumor to be assessed, the total area to be counted and which phase of mitoses constitutes a mitotic figure to be enumerated. The goal of counting mitotic figures in tumors is not to find the average number of mitoses, but the number of mitoses in a selected region of a tumor that correlates with prognosis and treatment selection.

**Recommendation for MC:** Scan the slide and select a cellular region at the periphery of the tumor where the most mitotic activity is seen. The region recommended is at the periphery of the tumor margin with nonneoplastic tissue, an area representing the

invasive front, where fixation is typically optimal. Count mitotic figures in  $2.37\text{mm}^2 = 10$  contiguous, no overlapping FOVs, avoiding and/or skipping areas of the tumor that are cell poor from hemorrhage, edema, necrosis, cysts etc. Count only definitive mitotic figures: nuclear aggregates that do not have a nuclear membrane (passed prophase) that have definite hairy projections of nuclear material (chromosomes) that are a single aggregate (metaphase), in a plane (metaphase/anaphase) or in two distinct aggregates (telophase). Telophase should be counted as one, however, automated counting methods and artificial intelligence may count telophase as two. Pyknotic, hyperchromatic and doubtful structures should not be counted. Other parameters that should be standardized are: margins, necrosis, recurrence, metastases and outcome assessment.

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### **CLINICAL, HISTOPATHOLOGIC, AND IMMUNOHISTOCHEMICAL FEATURES OF THIRTEEN CASES OF CANINE GALLBLADDER NEUROENDOCRINE CARCINOMA**

Braelyn Bankoff, Kevin O'Brien, Peri Rosenstein, Daphne Clendaniel, Melissa Sánchez, Amy Durham

**Background:** There are few cases of canine gallbladder neuroendocrine carcinomas (NEC) in the literature with little known about its clinical features.

**Objective:** The aim of this retrospective study is to characterize the clinical, histologic, and immunohistochemical features of canine gallbladder NEC.

**Methods:** 13 cases of gallbladder NEC were diagnosed at PennVet Diagnostic Laboratory and reviewed for histologic characteristics, vascular invasion, and margins. Immunohistochemical stains for neuroendocrine markers (neuron specific enolase (NSE), chromogranin A, synaptophysin) and gastrin were evaluated, and clinicopathologic and follow-up data obtained for all cases.

**Results:** Breeds include Boston terrier (6/13), bichon frise (2/13), poodle (1/13), English bulldog (1/13), French bulldog (1/13) and mixed breed (2/13). Boston terriers were overrepresented in this cohort; therefore, a breed predilection is possible. The average age at diagnosis was 8.9 years. The majority of dogs presented with emesis and elevated liver enzymes: 13/13 elevated alanine aminotransferase (ALT) and alkaline phosphatase (ALP); 8/13 elevated aspartate aminotransferase (AST); 7/13 elevated gamma-glutamyl transferase (GGT). Abdominal ultrasound and/or exploratory surgery revealed a gallbladder mass. All neoplasms have similar histologic features and positive immunoreactivity for NSE, chromogranin A, synaptophysin and gastrin. Vascular invasion was noted in 8/13 neoplasms (62%) and metastasis was histologically confirmed in 6/13 cases (46%); 4 hepatic and 2 pulmonary metastases. Seven dogs (54%) are deceased and the median survival time was 3.5 years; 4/7 deaths were directly attributed to the gallbladder NEC.

**Conclusions:** Gallbladder neuroendocrine carcinomas have the potential to metastasize; however, surgical excision may be curative in a subset of dogs.

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## **IMMUNOHISTOCHEMICAL DIAGNOSIS AND CHARACTERIZATION OF THE IMMUNE CELL POPULATION IN CANINE CHOROID PLEXUS TUMORS**

Martha Dalton, Justin Stilwell, Andrew Miller, Paula Krimer, Daniel Rissi

**Background:** Choroid plexus tumors (CPTs) are classified as papilloma (CPP, grade I), atypical CPP (ACPP, grade II), and carcinoma (CPC, grade III). CPCs can disseminate via cerebrospinal fluid and be mistaken for a metastatic carcinoma. Kir7.1 immunohistochemistry (IHC) is a reliable tool for diagnostic confirmation of CPTs and differentiation from metastatic carcinomas.

**Objective:** To characterize the immune cell population of canine choroid plexus tumors.

**Methods:** Archived tissue sections were examined and immunolabelled with Kir7.1 for diagnostic confirmation. IHC for CD3, CD20, MAC387, and Iba1 was performed for immune cell characterization. The number of stained inflammatory cells for each antibody was counted in ten 400x fields and individual cumulative values were generated. Spearman's rank correlations evaluated relationships within IHC values and tumor grades.

**Results:** Kir7.1 immunoreactivity was detected in all cases, and was intense at the apical cell membrane in CPPs and ACPPs and intense at the apical cell membrane and cytoplasm in CPCs. CD3<sup>+</sup> lymphocytes and CD20<sup>+</sup> lymphocytes were present within and around tumors (11/11 cases) and trended together ( $p=0.005$ ). Intravascular MAC387 immunoreactivity occurred in 5/11 cases and Iba1 immunoreactivity was robust and observed within and around tumors in 11/11 cases.

**Conclusions:** This study supports the reliability of Kir7.1 for diagnostic confirmation of canine CPTs. While immune cells were present in all cases, no significant associations were found between the type of cells and tumor diagnosis. The characterization of the immune cell infiltration within CPTs could be useful in future implications of immunotherapy.

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## **P63/P40 IMMUNOREACTIVITY IN A CANINE PULMONARY SQUAMOUS CELL CARCINOMA**

Jason Crawford, Terri Franks, Brandon Culbertson, Derron Alves

**Background:** This 10-year-old, female spayed, Labrador retriever was euthanized after a three-month history of a progressive cough, unresponsive to antibiotics-anthelmintics. Effacing the right caudal lung lobe was a pale-tan solitary, 11.0 x 8.0 x 4.0 cm, mass with multifocal, 1.0 to 5.0 cm diameter, gray, air-filled bullae throughout all lung lobes and a lobulated, 11.0 x 8.0 x 4.0 cm, mediastinal mass. Both masses were fixed in formalin and submitted for histopathology.

**Methods:** Representative samples from the mediastinal and pulmonary masses were processed routinely, embedded in paraffin, and stained with H&E. Both masses were stained with immunohistochemistry antigens for thyroid transcription factor-1 (TTF-1), P63, P40, and pancytokeratin (AE1/AE3).

**Results:** Neoplastic polygonal cells form broad cords and vague acini with squamous differentiation. Neoplastic cells express strong nuclear immunoreactivity for both P63 and P40; 10% of neoplastic cell were immunoreactive to pancytokeratin; neoplastic cells were non-immunoreactive to TTF-1. These findings are consistent with primary pulmonary squamous cell carcinoma (SCC).

**Conclusions:** Primary pulmonary SCC is uncommon in veterinary medicine. These aggressive epithelial neoplasms are usually solitary masses and exhibit intrapulmonary and lymphatic metastasis. P63 is a highly sensitive marker reported in human SCC, yet exhibit immunoreactivity in 31% of pulmonary adenocarcinomas. P40 labeling was reported in only 3% of adenocarcinomas; making P40 a highly specific marker, differentiating SCC from adenocarcinoma. Positive immunoreactivity to P63/P40 with negative immunoreactivity to TTF-1, increases the specificity of diagnosing pulmonary SCC. To this author's knowledge, this is the first reported use of P40 to diagnosis canine pulmonary SCC.

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2:50 PM – 3:00 PM

#### **PRIMARY CENTRAL NERVOUS SYSTEM LARGE GRANULAR LYMPHOCYTE (LGL) LYMPHOMA IN A YOUNG ADULT DOG**

Taylor Towns, Eric Fish, Maninder Sandey, Amy Yanke, Jey Koehler

**Clinical case:** A 4.5-year-old, female spayed, Siberian Husky dog presented for evaluation of progressively-worsening seizure activity over a 6-month period. On magnetic resonance imaging of the brain, right cerebral abnormalities consisted of irregularly marginated intra-axial T2 hyperintensity with associated mass effect, midline shift and contrast enhancement of the pachy- and leptomeninges; an underlying neoplastic etiology (round cell tumor or histiocytic sarcoma) was suspected. Analysis of cerebrospinal fluid (CSF) revealed a mildly increased nucleated cell count (20/uL) and microprotein (99 mg/dL). CSF cytology contained large neoplastic lymphocytes measuring ~12-24 um with distinct red intracytoplasmic granules. The diagnosis was neoplastic pleocytosis with large granular lymphocyte (LGL) lymphoma. The patient was euthanized, and at necropsy there were several, 1.0-cm, variably distinct grey nodules within the right cerebral cortex. Histologically, these were nonencapsulated, infiltrative, poorly circumscribed and densely cellular neoplasms composed of round cells that had strong membranous immunoreactivity to CD3 antigen, and no immunoreactivity for Pax5 and CD79a antigens. PTAH-positive intracytoplasmic granules were present in some cells. Lymphoma was not observed grossly or histologically outside of the brain. A final diagnosis of primary CNS T-cell lymphoma was made, with evidence of granular/LGL morphology on both the CSF cytology and histopathology of the mass.

**Discussion:** In human and canine patients, LGLs have been proposed to arise from either cytotoxic T lymphocytes or natural killer (NK) cells. The putative cell of origin in this case are cytotoxic T lymphocytes. This is the first report of primary CNS LGL lymphoma in a young adult dog.

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### **ENDOCARDIOSIS IN TETRAS (FAMILY: CHARACIDAE)**

Robert Kim, Andrew Cartoceti, Judy St. Leger, Roy Yanong, Kali Holder, Elise LaDouceur

**Background:** Endocardiosis has been described in zebrafish (*Danio rerio*, family: Cyprinidae), and reported anecdotally in Atlantic salmon (*Salmo salar*, family: Salmonidae), in which the significance of this disease is unknown. This study describes endocardiosis in tetras (family: Characidae) with comparisons to published lesions of endocardiosis in zebrafish.

**Methods:** Necropsy records of tetras from two institutions were searched for cardiac lesions consistent with endocardiosis. Five cases were identified and histology was reviewed. Control tetras (*Paracheirodon innesi*) were also evaluated histologically.

**Results:** Endocardiosis was identified in five tetras (four *Paracheirodon innesi* and one *Hemigrammus rhodostomus*) that were at least 8 months old. Four tetras were found dead, and one was euthanized for coelomic distention. Histologically, proliferative myxomatous matrix expanded multiple cardiac valves (valvular endocardiosis) and/or cardiac wall (mural endocardiosis), and frequently occluded a substantial portion of cardiac chamber lumina in the sections examined. Endocardiosis was considered the cause of death in four tetras due to severity of endocardiosis and lack of identifiable comorbidities. One tetra had concurrent egg binding, and this case had the least severe endocardiosis, which was considered a comorbidity, but not necessarily the cause of death. Retrospective review of tetra submissions from two institutions suggested a prevalence of endocardiosis in tetras of 5.7% and 4.3%, respectively.

**Conclusions:** In zebrafish, endocardiosis lesions ranged in severity, but were uniformly substantially less severe than these lesions in tetras. Endocardiosis is a previously unreported contributor to mortality in tetras, in which this disease can cause nearly complete occlusion of cardiac chambers.

November 6, 2018

3:40 PM – 3:50 PM

### **CORRELATING HEPATIC HISTOPATHOLOGY TO GALLBLADDER DISEASE IN DOGS**

Eunju Choi, Sean McDonough

**Background:** The spectrum of liver pathology associated with gallbladder disease in the dog has not been well characterized.

**Objective:** To document hepatic pathology related to concurrent gallbladder disease.

**Methods:** The histologic features of livers and paired gallbladders are compared in 33 dogs.

**Results:** The most common gallbladder disease was a mucocoele (14/33) and 6/33 had features of an emerging mucocoele. Of the 14 mucocoeles, 4/14 had rupture with granulation tissue formation and serositis and 2/14 had histologically visible intraluminal bacteria. Four cases of hemobilia were associated with: bacterial cholecystitis (1/4), chronic toxic hepatopathy (1/4), gallbladder microliths with mural granulation tissue (1/4), and suppurative cholangiohepatitis (1/4). Two cases of necroulcerative cholecystitis were associated with intraluminal bacteria (1/2) and acute hepatotoxicity (1/2). The most common histopathologic finding with any gallbladder disease was bile duct dilation and luminal mucin stasis (32/33), followed by ductular reaction (21/33). Bacteria were identified in 6/33 gallbladders, including the 2 mucocoeles, of which 5/6 had corresponding suppurative cholangitis/cholangiohepatitis and 1/6 had lymphoplasmacytic cholangitis suggestive of chronic bacterial infection. Seven cases of suppurative cholangitis/cholangiohepatitis did not have evidence of bacterial infection in the gallbladder.

**Conclusions:** Gallbladder disease can be associated with a range of histologic features in the liver irrespective of the etiology. Approximately half of the dogs with suppurative cholangitis/cholangiohepatitis had gallbladder involvement either in the form of suppurative cholecystitis or intraluminal bacteria. Gallbladder diseases may be considered when there are subtle features in the intrahepatic biliary tree such as bile duct dilation, mucin stasis, and ductular reaction.

November 6, 2018

3:50 PM – 4:00 PM

### **GLOBOID CELL LEUKODYSTROPHY IN THREE RELATED BORDER COLLIE MIXED-BREED PUPPIES**

Caitlin Burrell, Miranda Vieson, Devon Hague, Barbara Kompare

**Background:** Three five-month-old, related, Border Collie mixed-breed puppies presented for necropsy due to progressive ataxia, incontinence, and head tremors. Antemortem PCR testing for Imlerslund-Gräsbeck syndrome and serologic testing for *Toxoplasma gondii* and *Neospora caninum* was negative and cobalamin levels were high. MRI of the brain and spinal cord of one puppy indicated symmetrical cortical and cerebellar white matter hyperintensity and contrast enhancement, suggestive of leukoencephalopathy due to an inflammatory or metabolic disease process. Treatment with doxycycline, clindamycin, and prednisone was unsuccessful. During gross examination, no lesions were identified in the central or peripheral nervous systems. On histopathology, the white matter and perivascular spaces in the brain and spinal cord contained numerous plump macrophages with abundant amphophilic fibrillar to flocculent cytoplasm (globoid cells).

**Objective:** Our objective was to identify “globoid cells” within the brain and spinal cord white matter and confirm the diagnosis of globoid cell leukodystrophy in a previously unreported canine breed mix.

**Methods:** A gross examination was completed on all three puppies. Formalin-fixed, paraffin-embedded sections of the cerebrum, cerebellum, midbrain, spinal cord, and peripheral nerves were stained with hematoxylin and eosin.

**Results:** Globoid cells were present within the white matter and perivascular spaces of all three puppies. Cellular cytoplasm stained positively with Periodic Acid-Schiff stain. Globoid cells were not identified in the peripheral nerves.

**Conclusions:** The distribution and morphology of the globose macrophages in the central nervous system is consistent with globoid cell leukodystrophy, an autosomal recessive lysosomal storage disease. This report documents this condition in three related puppies.

November 6, 2018

4:00 PM – 4:10 PM

### **AORTIC LESIONS IN TWO WILD COMMON BOTTLENOSE DOLPHINS (*Tursiops truncatus*)**

David Rotstein, D Pabst, William McClellan, Tiffany Keenan-Bateman

Aortic disease in marine mammals is uncommon and includes stingray barb-associated rupture, congenital anomalies, and *Crassicauda* spp thoracic aorta and renal artery migration. In January 2018, two common bottlenose dolphins (*Tursiops truncatus*) were stranded within 8 weeks along the southeastern North Carolina coast. Dolphin 1 was a 240.0 cm and 206.6 kg, adult pregnant female in robust body condition. Primary gross findings were a circumferential, regular linear tear in the aorta that extended nearly the entire aortic circumference and hemopericardium. The stomach was full of fish. Histopathologic findings in the aorta included separation of the lamina, which was filled with fibrin, extravasated erythrocytes, fibrin, and small numbers of neutrophils. Death was attributed to hypovolemia and cardiac tamponade. A cause was not evident, but pregnancy may have been a factor based on reported aortic dissections during pregnancy in women. Dolphin 2 was a 268.0 cm and 241.0 kg, adult male in good body condition. The primary gross finding was an approximately 1 cm diameter irregularly subcircular tear in an aneurysmal dilation off of the aorta and hemopericardium. Histopathologic findings were observed in the cardiovascular, urogenital, nervous, and respiratory system. There was a neutrophilic aortitis, pulmonary arterial thrombosis, epicarditis, and myocardial fibrosis. In addition, there was non-suppurative meningitis, epididymitis, and parasitic bronchopneumonia. PCR for *Brucella* spp. was positive and is the likely cause of aortitis. The cases involving infectious and non-infectious aortic lesions provide a more complete understanding of population health of cetaceans.

November 6, 2018

4:10 PM – 4:20 PM

### **PYOGRANULOMATOUS OTITIS MEDIA, MENINGOENCEPHALITIS & OSTEOMYELITIS IN A GOAT CAUSED BY *Rhodococcus equi***

Michael Cruz Penn, Ahmad Saied

A 21-month-old, female, Boer goat was submitted for necropsy at the UKVDL. The goat had a 5-day-history of malaise followed by neurological signs characterized by opisthotonus, nystagmus, and ataxia. Terminally, the goat became recumbent and was unable to stand, and the owner elected euthanasia. Grossly, the goat had an approximately 3 cm diameter mass in the right middle ear. The mass obliterated the tympanic bullae and extended into the occipital bone and meninges, compressing the cerebellum. Histological examination revealed marked, locally extensive pyogranulomatous otitis media, meningoencephalitis of the cerebellum, and osteomyelitis of the occipital bone. Bacterial cultures from the pyogranulomatous mass yielded numerous *Rhodococcus equi*. Polymerase chain reaction analysis of the isolate detected *choE* gene, which is used for rapid identification of *R. equi*. The bacteria also lack the circular plasmid; *vap A*, that is associated with virulence in horses. This is an interesting case of pyogranulomatous otitis media, meningoencephalitis and osteomyelitis in a goat due to *Rhodococcus equi* infection.

November 6, 2018

4:20 PM – 4:30 PM

#### **BRUCELLA OOPHORITIS IN A MARINE TOAD (RHINELLA MARINA)**

Raisa Glabman, Rinosh Mani, Dalen Agnew

A 4-year-old female marine toad (*Rhinella marina*) presented with an approximately 3 cm encapsulated mass on the left ovary, as well as a 1.5 cm subcutaneous mass. Routinely stained histopathologic sections from both the ovarian and subcutaneous mass revealed severe granulomatous inflammation. Lesions were characterized by multifocal to coalescing areas of necrosis surrounded by sheets of macrophages mixed with scattered lymphocytes and heterophils. Surrounding necrotic areas were large numbers of reactive fibroblasts and epithelioid macrophages interspersed by bands of collagen. Within the cytoplasm of macrophages, and freely within the foci of necrosis were numerous Gram negative, PAS negative, and acid fast negative coccobacilli. *Brucella* spp. were cultured from fresh tissue collected from both the female and a male from the same shipment and identified to the genus level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Partial 16S rRNA sequencing was performed and confirmed *Brucella inopinata*-like spp. Recently, 'atypical' *Brucella* strains have been isolated from non-mammalian hosts including amphibians. The precise epidemiology and pathogenesis of *Brucella* in amphibians remains largely unknown, and limited data is available for the zoonotic potential of novel or 'atypical' members of the *Brucella* genus. As such, this finding represents a potentially novel emerging disease in toads and zoonotic pathogen.

November 6, 2018

4:30 PM – 4:40 PM

#### **PROLIFERATIVE REPRODUCTIVE TRACT LESIONS IN A FEMALE AFRICAN GREEN MONKEY (CHLOROCEBUS AETHIOPS SABAEUS)**

Gayathriy Balamayooran, Hannah Atkins, Matthew Jorgensen, J Cline, Nancy Kock

We report here three proliferative lesions in the reproductive system of a 25 year old, multiparous female vervet. On gross examination, the endometrium was roughened and

irregularly thickened up to 5mm, the left ovary was atrophied, the right ovary was not found, and the right oviduct was irregularly enlarged. On histology, the endometrial changes ranged from complex hyperplasia to low-grade carcinoma, with compression of adjacent glands, low numbers of mitoses, focal atypia, focal cribriform change, and focal invasion. In addition, a leiomyoma was present in the myometrium. The proliferative endometrial cells displayed intense nuclear staining for both estrogen (ER) and progesterone receptors (PR) but minimal to no staining for tumor suppressor gene p53. The leiomyoma stained positive for ER and PR, and the ER staining was much more intense compared to the normal myometrium. Microscopic examination of the right oviduct disclosed segmental hyperplasia and atypia resembling serous tubular intraepithelial carcinoma. The proliferative oviductal epithelium had segmental positive staining for ER, PR, and p53. Serum estradiol at the time of death was undetectable at < 5 pg/ml, however, the presence of ER and particularly the downstream ER-dependent marker PR, suggests that estrogen-mediated pathways were active in all lesions. Overexpression of p53 in the oviductal lesion may reflect a similar pathogenesis to that reported in women with serous tubular intraepithelial carcinoma. To the authors' knowledge, this is the first report of neoplastic proliferative endometrial and oviductal lesions in a vervet monkey.

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November 6, 2018

4:40 PM – 4:50 PM

## **CLINICAL AND HISTOPATHOLOGIC FEATURES OF CANINE PERIPHERAL ODONTOGENIC FIBROSARCOMA (ODONTOGENIC SARCOMA)**

Inshil Kim, Melissa Sanchez, Mary Volker

**Background:** There are few cases of peripheral odontogenic fibrosarcomas (odontogenic sarcomas) in the literature with little known about its clinical features.

**Objective:** The aim of this retrospective study is to characterize the clinical and histologic features of peripheral odontogenic fibrosarcomas (odontogenic sarcomas) in dogs.

**Methods:** Nine dogs were identified with a diagnosis of odontogenic sarcoma or periodontal fibrosarcoma, herein referred to as peripheral odontogenic fibrosarcoma, based on excisional biopsy at the Animal Dental Center. Inclusion criteria included complete medical records with photographs, imaging, surgery, histopathology, and follow-up information.

**Results:** Patient age ranged from 2-11 years. The neoplasms were located both on the maxilla and mandible. Imaging findings included osteolysis, external inflammatory root resorption, displacement and/or extrusion of associated dentition, and osteoproduction within the mass. Peripheral odontogenic fibrosarcomas were histologically low-grade, characterized by hypercellularity of the spindle cell component, low mitotic activity, variable presence of odontogenic epithelium, and variable presence of hard substance

(cementum/dentinoid/bone). All patients had complete surgical resection of the masses. Recurrence was not observed after complete surgical excision (at least 6 months follow-up). Metastasis was not observed.

**Conclusions:** Peripheral odontogenic fibrosarcomas (odontogenic sarcomas) are occasionally locally invasive counterparts of peripheral odontogenic fibromas (former fibromatous epulis of periodontal ligament origin). These neoplasms are amenable to complete surgical excision. This clinical study is submitted for publication in the Journal of Veterinary Dentistry.

November 6, 2018

4:50 PM – 5:00 PM

### **MIRNA GLOBAL GENE EXPRESSION IN CANINE PRIMARY MAMMARY CARCINOMA AND IN THE RESPECTIVE LYMPH NODE METASTASIS**

Renee Laufer-Amorim, Rosana Salvador-Bernabé, Sandra Drigo, Marcio Carvalho, Felipe Sueiro, Elan Paes de Almeida

**Background:** Canine mammary tumors are the most common tumor in female dogs and it shows great relevance in Veterinary Oncology. Metastasis to the regional lymph node is a prognostic factor that negatively impacts the survival time of these patients. In canine mammary tumors there are few studies evaluating miRNAs.

**Objective:** This study aimed to identify deregulated miRNAs in lymph node metastasis in comparison with paired canine mammary primary tumors.

**Methods:** Global expression profile of miRNAs in paired canine mammary carcinoma samples and their metastatic lymph nodes were evaluated using the Affymetrix high-density microarray platform. Twenty-six samples of metastatic mammary carcinomas (MMC) and 26 samples of their respective regional lymph node metastasis (MMC\_Met) were analyzed.

**Results:** Ten miRNAs (miR-199a-3p, miR-199b-3p, miR-205-5p, miR-199a-5p, miR-195-5p, miR-152-3p, miR-497-5p, miR-27a-3p, miR-99a-5p and miR-27b-3p) were detected as differentially expressed (all down-regulated) in MMC\_met compared with their primary tumors. The miR-205-5p, presenting the lowest fold change (FC=-27.03, P=0.001), was further confirmed by RT-qPCR. In silico analysis identified 120 target genes regulated by miRNA-205-5p. Enrichment analysis revealed that these genes participate in different pathways, such as cell adhesion, cell cycle, miRNA in cancer, PI3K-AKT signaling pathway, p53 and focal adhesion.

**Conclusion:** Like in humans, down-regulation of miRNA-205-5p can be associated with metastatic progression of canine mammary tumors. In addition, downregulation of miRNA-205-5p in metastatic lesions can modulate genes involved in cancer-related pathways. These results may guide future clinical studies evaluating this miRNA as a prognostic tool in metastatic mammary gland tumors in dogs.

Financial support: Fapesp and CNPq



## Diagnostic Pathology Focused Scientific Poster Session

### D-001: TRAUMATIC LINGUAL ENTRAPMENT AND GRANULOMA FORMATION IN A CAPTIVE CUBAN BOA (*EPICRATES ANGULIFER*)

Jason Thornton, Erin Ball, Michael Garner, Brittany Marble

**Background:** An adult male Cuban boa (*Epicrates angulifer*) presented with progressive weight loss, lethargy, and anorexia of four months duration. Clinical examination and radiographs revealed a firm, well-circumscribed, 6x6x3 cm, oblong, subcutaneous mass adhered to the base of the caudal tongue and obstructing the esophagus. The mass was surgically excised and submitted for histologic evaluation.

**Methods:** The tissue was fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections of the mass were stained with hematoxylin and eosin (HE). Histochemical stains were performed to screen for bacteria and fungi.

**Results:** Grossly, the submitted tissue was composed of laminated tan friable material surrounding a 1.5x1.5x1 cm section of tongue and bound by a dense fibrous capsule. Histologically, abundant necrotic cellular debris admixed with numerous degenerate heterophils, epithelioid macrophages, and multinucleated giant cells expanded the space between the tongue and the glossal sheath, causing entrapment of the tongue. The epithelium of the glossal sheath was hyperplastic, dysplastic, and subtended by fibrous connective tissue. Gram stains revealed mixed colonies of gram-positive and gram-negative bacteria.

**Conclusions:** The radiographic, gross, and microscopic findings are consistent with a granuloma surrounding segments of the caudal tongue with entrapment by the glossal sheath. We suspect a portion of the tongue may have been traumatically cleaved and entrapped or partially swallowed during feeding, with subsequent overgrowth of commensal oral bacteria and a profound granulomatous response that occluded the rostral esophagus. As of this report, the snake is doing well and eating normally following surgical excision.

### D-002: MALIGNANT COLONIC GLOMANGIOMA IN AN ADULT THOROUGHBRED HORSE WITH CONCURRENT *CLOSTRIDIUM DIFFICILE*

Emily Brinker, Tamara Sierra R., Claudia Reyner, Jey Koehler

**Background:** A 15-year-old Thoroughbred gelding was presented for postmortem examination following an approximately 24-hour history of sanguineous diarrhea. Despite intensive medical therapy, the horse developed septic shock and encephalopathy.

**Objective:** Our objective was to determine the underlying pathology of the hemorrhagic diarrhea. We also sought to characterize the colonic and hepatic tumors observed grossly during postmortem examination.

**Methods:** Samples were examined histologically and feces were submitted for *Clostridium* toxin typing. Immunohistochemistry was performed on sections of colon with

alpha-smooth muscle actin, desmin, and c-kit. Gram and Alcian Blue stains of sections of colon were performed.

**Results:** On routine histology, the colonic mass was expanding the submucosa and forming a non-encapsulated and multilobulated, densely cellular mass composed of spindle cells arranged in an organoid pattern along abundant dilated blood vessels. Neoplastic cells had cytoplasmic immunoreactivity for alpha-smooth muscle actin, multifocal cytoplasmic immunoreactivity for desmin, and lacked immunoreactivity for c-kit. There was regional colonic metastasis and hepatic metastasis of the neoplasm. Throughout the colon, there was severe diffuse necrohemorrhagic colitis. Feces were positive for *C. difficile* toxins A and B.

**Conclusion:** The colonic neoplasm most resembles a malignant glomangioma, a morphological variant of glomus cell tumor, of which only few cases have been reported in domestic animals. *Clostridium difficile* is a rare cause of diarrhea in an adult horse with no recent history of antibiotic administration. This case report expands the current literature of malignant glomus cell tumors in horses, as well as provides a possible predisposition to *Clostridial* enterocolitis.

#### **D-003: DETERMINING THE ORIGIN OF A CARCINOMA WITH PULMONARY AND GASTROESOPHAGEAL JUNCTION INVOLVEMENT IN A CAT**

Timothy Wu, Eunju Choi

**Background:** A 12-year-old male, castrated, domestic medium-haired cat presented for inappetence and anemia. On presentation, the cat was in respiratory distress and pleural fluid was noted in the chest. Baseline bloodwork, urinalysis, abdominal ultrasound, thoracic CT, and fine needle aspirate revealed anemia, disseminated intravascular coagulation (DIC), isosthenuria, and masses in multiple organs. Euthanasia was elected, and the body was donated for an educational necropsy.

**Objective:** To determine the origin of the carcinoma involving the lung and gastroesophageal junction with other metastatic lesions.

**Methods:** The cat was necropsied and slides were prepared from formalin fixed, paraffin embedded tissues, and stained with hematoxylin and eosin. Additional immunohistochemistry was performed on sections of mass following initial histopathologic examination.

**Results:** On necropsy, multiple white, firm, multinodular masses were seen throughout the body, including the gastroesophageal junction, lungs, pericardium, and intercostal muscles. Histopathology of these masses revealed a large population of polygonal cells arranged in tubules, islands, and large coalescing cystic structures lined by stratified epithelium, compatible with an adenocarcinoma. The neoplasm had striking angioinvasive features that correlated with lung lobe infarction and cardiac fibrosis. Immunohistochemistry revealed immunoreactivity to cytokeratin (CK) 7 and CK19, and no immunoreactivity to CK20.

**Conclusions:** Based on the presenting location and size of the masses, an esophageal adenocarcinoma was favored over a pulmonary adenocarcinoma. To our knowledge, this case represents the first report of a suspected esophageal adenocarcinoma in a cat.

#### **D-004: LIPOGRANULOMATOUS ENTERITIS IN A SPOTTED MORAY EEL (GYMNOTHORAX MORINGA)**

Jacqueline Elliott, Jamie Torres, Dawn Evans

**Background:** A male spotted moray eel (*Gymnothorax moringa*) of unknown age and in good body condition was found dead on exhibit at the Audubon Aquarium of the Americas in New Orleans, Louisiana. A necropsy was performed on site, and gross findings reported included a mottled liver and marked erythema and inflammation of the gastrointestinal tract.

**Objective:** Our objective was to identify a cause of death in this moray eel via histopathological evaluation.

**Methods:** Select formalin-fixed tissues were submitted to the Louisiana Animal Disease Diagnostic Laboratory at Louisiana State University for histological evaluation.

**Results:** On gross examination, intestinal sections have numerous thick yellow villous projections. Histopathologic findings include sheets of vacuolated polygonal cells expanding the intestinal lamina propria. These cells are positive with Oil Red O for lipid. Sections of the stomach have infrequent granulomas with the submucosa, along with low numbers of lymphocytes and plasma cells surrounding blood vessels.

**Conclusions:** A definitive cause of death was not identified. Lipid accumulation is presumed to be similar to abnormal cases described in the literature in eels including ocular lipid keratopathy and xanthomatous panniculitis. After consultation with other aquatic diagnosticians and pathologists, the exact pathological significance of this lesion is yet to be determined.

#### **D-005: MYCOBACTERIAL INFECTION IN A PET ZEBRA FINCH**

Kristen Hill-Thimmesch, Tsang Long Lin, Tamartha Hickok, Pat Wakenell

A 6-year-old, male, Zebra Finch was humanely euthanized following a two-week history of weakness and respiratory distress. These symptoms were characterized by unwillingness to perch, lethargy and an increased respiratory rate. Gross examination revealed few, scattered, white to tan nodules adhered to the visceral aspect of the sternum and right rib cage. Identical nodules were on the left liver lobe and pericardial sac adjacent to the base of the heart. Histopathologic examination of the heart, lung, air sac, esophagus, crop, liver, spleen, skeletal muscle, testicle and kidney found multifocal to coalescing infiltrates of plump, round, pale basophilic macrophages measuring 25-30um in diameter with abundant, foamy cytoplasm. Histochemical stains (Periodic acid-Schiff, Gram and Ziehl-Neelsen) were performed in selected tissue sections. Periodic acid-Schiff stain was negative for fungi. Gram stain revealed weakly Gram positive bacilli in macrophages. Ziehl-Neelsen stain was positive for numerous acid-fast bacilli

within macrophages. Based on the granulomatous inflammation combined with the presence of acid-fast bacteria, a diagnosis of avian mycobacterial infection was made. These mycobacterial organisms are present in the environment and cause sporadic incidents in backyard poultry, game birds and captive birds.

#### **D-006: EXOCRINE PANCREATIC ADENOCARCINOMA IN A TURKEY (MELEAGRIS GALLOPAVO)**

Alex Byas, Gretel Tovar Lopez, Matthew Johnston, Lukas Kawalilak, Jonathan Plenn, Juan Muñoz Gutiérrez

**Case Report:** A four year old, female pet turkey presented to the Colorado State University Veterinary Teaching Hospital for lethargy and open mouth breathing. Physical exam revealed cyanotic mucous membranes and coelomic fluid upon palpation. Radiographs showed loss of serosal detail within the coelomic cavity and compression of the caudal thoracic and abdominal air sacs. Ultrasound revealed multiple coelomic masses and coelomic fluid. The turkey died during treatment and was submitted for postmortem examination. At necropsy there were multiple firm, tan, lobular, cerebriform masses which effaced the pancreas, ovary, oviduct, mesentery and intestinal serosal surfaces. There was also hemocoelom and multiple variably developed follicles located free within the coelom. Histologically these masses were a well-differentiated exocrine pancreatic adenocarcinoma characterized by formation of acinar structures, abundant scirrhous reaction, carcinomatosis, and frequent vascular invasion.

**Discussion:** On gross examination, ovarian/oviductal adenocarcinoma was considered most likely given its frequency in mature chickens and occasional reports in turkeys. However, this case was histologically determined to be a well-differentiated exocrine pancreatic adenocarcinoma with marked carcinomatosis. Exocrine pancreatic adenocarcinoma is uncommon in all domestic animals. Recognition of this entity in turkeys is diagnostically important as backyard birds have become more popular in the United States, and are sometimes kept until ages at which neoplasia is a common occurrence.

**Conclusion:** This is the first report of exocrine pancreatic adenocarcinoma in a turkey. This neoplasm should be considered as a clinical differential for neoplasia in aged turkeys.

#### **D-007: ROUND CELL NEOPLASIA IN PSITTACINE BIRDS: A DESCRIPTION OF MORPHOLOGICAL PRESENTATION**

Daniel Gibson, Nicole Nemeth, Hugues Beaufrère, Csaba Varga, Michael Garner, Leonardo Susta

**Background:** Round cell neoplasms, such as lymphoma, leukemia and histiocytic sarcoma are frequently diagnosed in a variety of avian taxa. In psittacine birds, round cell neoplasia is sporadic and there are limited data regarding prevalence and diagnostic features of these tumors. Lack of these criteria makes diagnosis challenging,

and restricts the formulation of reliable grading schemes to be used in prognostic studies.

**Objectives:** 1) To describe the demographic, anatomic, and histological characteristics of round cell neoplasia in a cohort of psittacine birds. 2) Establish a preliminary classification scheme for these tumors.

**Methods:** Cases of psittacine birds diagnosed over the last 20 years with round cell neoplasia were retrieved from the Ontario Veterinary College and other North American institutions. Slides were reviewed for organ distribution of the neoplasm, growth pattern, and cellular morphology (including mitotic index).

**Results:** Fifty-one cases of birds with a median age of 4 years were identified in 12 psittacine species. Cockatiels (41%) and budgerigars (21%) made up the majority of the cases. Most cases (76%) were diagnosed as lymphoma. Tumors were mainly infiltrative and multicentric, and only rarely presented as expansile masses involving a single organ. Microscopically, cells formed homogenous sheets of round to polygonal cells, had scant cytoplasm, and mitotic rate varied from 0 to 30 per high-power field.

**Conclusions:** This is the first study describing a large cohort of round cell neoplasia in psittacine birds, and provides preliminary information to increase the accuracy of diagnosis to better characterize these diseases.

#### **D-008: A CASE OF INCLUSION BODY HEPATITIS IN TEN WHITE BROILERS (*GALLUS GALLUS DOMESTICUS*) IN TAIWAN**

Ji-Hang Yin, Ying-Chen Wu, Hue-Ying Chiou, Jui-Hung Shien

**Background:** Inclusion body hepatitis (IBH) has been reported as viral disease, mainly associated with Aviadenovirus infection, and predominately affects commercial and farm birds.

**Case Description:** Ten 2-week-old chicks (*Gallus gallus domesticus*) from a commercial flock were presented to the Animal Disease Diagnostic Center, National Chung Hsing University for post-mortem examination with reported approximately two-thousand chicks found dead per day for four consecutive days. Due to the continuing sudden death and lack of response to medical therapy, the chicks were humanely euthanized for disease diagnosis.

**Results:** Grossly, the livers were invariably diffusely enlarged, pale, firm and friable with multifocal to coalescing, mottled from red to tan, pinpoint to 0.1 x 0.2 cm smooth areas on the surface and the parenchyma on cut surface. Skeletal muscle surrounding the femur, tibia and fibula had multifocal to coalescing, red to dark red, pinpoint, plaque to irregular areas (hemorrhage). Histopathologic examination revealed severe multifocal to coalescing necrotizing hepatitis foci rimmed by the hepatocytes containing small eosinophilic surrounded by a halo and large basophilic intranuclear inclusion bodies. No causative agents were cultured. Polymerase chain reaction (PCR) identified Aviadenovirus as the cause.

**Conclusion:** We herein present a case of inclusion body hepatitis in ten chicks based on the sudden onset death, characteristic gross lesion in liver, presence of two types of intranuclear inclusion bodies and the amplification of Aviadenovirus. We suspected vertical transmission in this case played a substantial role; however, fecal-oral route also needs to be taken into consideration.

**D-009: YOUNG PIGEON DISEASE SYNDROME IN A DOMESTIC PIGEON (COLUMBA LIVIA DOMESTICA)**

Yen-Chi Chang, Cheng-Hsin Shih, Jiunn-Wang Liao, Hue-Ying Chiou

A 3-week-old, female, domestic pigeon was presented to the Animal Disease Diagnostic Center of National Chung Hsing University for post-mortem examination with a history of lethargy and diarrhea for three days prior to being found dead. At necropsy, all lobes of the liver contain multifocal to coalescing tan to white spots, ranging from 0.1 - 1.0 mm in diameter. No gross lesion was observed in the bursa of Fabricius. Microscopically, necrotic bursitis, severe lymphoid depletion and massive amounts of botryoid, basophilic, intracytoplasmic inclusion bodies were observed in the bursa of Fabricius. Multifocal to coalescing necrosis of hepatocytes, deeply eosinophilic inclusion bodies with a clear zone between the inclusion and marginated chromatin and lightly basophilic inclusion bodies filling the nucleus were observed in the liver. DNA extracted from the liver and bursa of Fabricius were used for molecular biological examination of columbid herpesvirus-1 and pigeon circovirus, respectively. Both PCR results were positive. Bacterial isolation from the liver and spleen was attempted; however, there was no significant bacterial growth. The final diagnosis was young pigeon disease syndrome, a multifactorial disease in which the pigeon circovirus plays an important role in inducing immunosuppression followed by secondary infections.

**D-010: MYELOPROLIFERATIVE NEOPLASM AND AVIAN POXVIRUS INFECTION IN A CRESTED SERPENT EAGLE (SPILORNIS CHEELA)**

Yi-Hui Su, Ji-Hang Yin, Jiunn-Wang Liao

A subadult, female, weak crested serpent eagle (*Spilornis cheela*) was found in September 2014, and was sent to the Endemic Species Research Institute for medical care. During the treatment, the eagle showed abnormality in the right wing and was unable to fly. On November 13th 2016, the eagle showed sign of weight loss, swollen legs, multiple yellowish nodules in oral cavity, wart-like lesions on feet and face, and a mass on right elbow. Few bacteria and fungi were found in the oral swab, and PCR showed positive for avian pox virus. The patient was euthanatized on November 25th 2016, and was submitted to National Chung Hsing University Animal Disease Diagnosis Center for necropsy. Gross lesions showed swollen liver and spleen, multiple yellowish nodules in digestive system, a yellowish lesion on lung, and five masses on body. Histopathological examination revealed myelocytes infiltrating in the liver, spleen, thyroid glands, intestine, tongue and masses. Most of the masses also showed lymphocytes and lymphoblasts infiltration. The cutaneous lesion collected from facial and digital skin showed hydropic degeneration and hyperkeratosis in epithelial cells, and intracytoplasmic eosinophilic inclusion bodies were also found. PCR showed negative for both herpesvirus nuclear acid and avian leucosis virus subtype J nuclear

acid. The final diagnosis was myeloproliferative neoplasm and avian poxvirus infection in a crested serpent eagle (*Spilornis cheela*).

#### **D-011: HIP IMPLANT FAILURE ASSOCIATED PIGMENTED GRANULOMATOUS REACTION IN A DOG**

Elizabeth Howerth, Jeffrey Phillips

The right rear leg and pelvic bones forming the coxofemoral joint were submitted for evaluation of a suspected proximal femur osteosarcoma. The dog had a history of femoral head and neck removal following failure of a hip implant with a cobalt chrome stem with polyethylene parts. The coxofemoral joint was filled with fibrous tissue with extensive black pigmentation; similar black pigment covered the surface of the proximal femur. Histologically, tissue in the coxofemoral joint was composed of fibrocartilage, dense collagenous tissue and accumulations of epithelioid macrophages and a few multinucleated giant cells that sometimes surrounded necrotic foci. Abundant non-birefringent spherical to needle-like black pigment of variable size (interpreted to be conventional metallic particles) was both extracellular, streaking the collagen, and within fibroblasts and macrophages. Some macrophages were filled with finer corrosion-type metallic particles admixed with gray birefringent globular and needle-shaped material or larger strands (suspected polyethylene micro- and macroparticles). Findings were consistent with a local adverse reaction to implant materials. Reaction to implant metal is referred to as metallosis, but adverse local tissue reactions to implant materials should be more fully characterized by histological pattern and particle content to help clarify the pathogenesis of implant failure. Tissue analysis revealed high levels of aluminum, chromium, and cobalt consistent with many types of implants. Although not seen in this dog, metals released from failed implants, particularly cobalt, can result in neurologic damage.

#### **D-012: NATURAL KLEBSIELLA PNEUMONIAE INFECTION IN 9 RHESUS MACAQUES: A SUMMARY OF CLINICAL AND PATHOLOGICAL FINDINGS FROM THE CNPRC FROM 2010-2015**

Rebekah Keesler, Angela Colagross-Schouten, Rachel Reader

**Background:** *Klebsiella pneumoniae* (Kp) is a ubiquitous organism found in the environment and as an upper respiratory or gastrointestinal commensal in humans and animals. Recently, human Kp infection has become a significant nosocomial risk, and has been associated with immunosuppression, hospitalization and treatment with multiple antibiotics. Human disease is characterized by liver abscesses, pneumonia, and bacteremia resulting in meningoenzephalitis and endophthalmitis. Kp in veterinary medicine is most often reported in dairy cattle and rarely in non-human primates. Reports of natural Kp infections in NHP, particularly rhesus macaques (*Macaca mulatta*), are scarce in the recent literature.

**Objective:** Our objectives were: to describe the clinical and pathological findings associated with natural Kp infections in our colony of predominantly outdoor-housed rhesus macaques; and to compare these findings with typical disease patterns and risk factors identified in humans.

**Results:** Within a 5 year period, nine rhesus macaques had gross and/or histologic lesions consistent with Kp infection and confirmation via culture and/or PCR. Four animals presented with rapidly progressive clinical signs, including hypothermia, respiratory distress and altered mentation, and one animal had long-term hospitalization for an unrelated illness with multiple antibiotic treatments. Four animals were found deceased without clinical signs. At necropsy, the findings included liver abscesses, pneumonia, and suppurative meningoencephalitis and endophthalmitis.

**Conclusion:** Natural infection with *Klebsiella pneumoniae* produces lesions in rhesus macaques similar to the disease patterns in humans and is associated with similar risk factors.

#### **D-013: CARDIAC RHABDOMYOMA IN A CHIHUAHUA PUPPY**

Nathan Helgert, Mee-Ja Sula

A 4-week-old intact male Chihuahua puppy was euthanized and submitted for necropsy following a brief period of lethargy, hematemesis, and hematuria. Grossly throughout the myocardium were multiple 1x1x1 to 4x2x2mm areas of myocardial pallor. The heart weighed 0.88% of body weight (adult normal is 0.7-1.2%).

Histologically, 80% of the interventricular septal myocardium was replaced by well-demarcated clusters of myocardiocytes with abundant vacuolated eosinophilic sarcoplasm, which occasionally extended from the nucleus to the sarcolemma in thin strands (spider cells). Vacuoles frequently contained granular material that stained pink when exposed to the periodic-acid Schiff reaction. The material was diastase-sensitive, consistent with glycogen. The surrounding myocardiocytes were occasionally necrotic and there was abundant interstitial fibrosis within areas of myocardiocyte loss.

Rhabdomyomas are rare benign neoplasms, or potentially hamartomas, of striated muscle origin with abundant glycogen accumulation. In veterinary medicine, they are most commonly reported as incidental lesions identified in swine at slaughter. In dogs, most reports occur within the laryngeal and pharyngeal regions. Rhabdomyomas are the most common cardiac neoplasm in children and are occasionally associated with tuberous sclerosis, which causes tumors in multiple organs due to mutations of TSC-1 and TSC-2 genes; this condition was not identified in the current case. No other underlying cause for the reported clinical signs was identified, and we propose that the amount of myocardium replaced by this neoplasm and the attendant fibrosis may have caused conductive and contractile deficits resulting in poor systemic perfusion and lethargy.

#### **D-014: CEREBRAL TISSUE PULMONARY EMBOLISM IN A PUPPY WITH SEVERE HEAD TRAUMA**

Miranda Vieson, Rachel Teixeira-Neto

**Background:** An embolus is an intravascular solid, liquid, or gaseous mass carried by blood to a site distant from its point of origin. Pulmonary embolism is often clinically silent, but can cause pulmonary hemorrhage, hypertension, right ventricular cardiac

failure, or sudden death in the event that 60% or more of the pulmonary circulation is obstructed.

**Objective:** This report investigates the origin and nature of pulmonary vascular emboli identified on post mortem examination of a canine with severe head trauma.

**Methods:** Post mortem examination of a 9-week-old, male, mixed breed dog revealed severe high-energy trauma to the head and pulmonary emboli with features of neural tissue. Further identification of the tissue embolism is investigated by immunohistochemistry for glial acidic fibrillary protein (GFAP) and neuron-specific enolase (NSE).

**Results:** Multiple pulmonary vessels are occluded by emboli characterized by a fine fibrillary eosinophilic meshwork studded by low numbers of individualized angular cells with features of neurons and small round cells with features of glial cells. Rare cells with glial features had scant granular cytoplasmic immunoreactivity for GFAP. The eosinophilic meshwork had moderate to strong granular immunoreactivity to NSE. In a portion of cerebellum, similar IHC-staining profiles and histomorphology were observed in the molecular layer.

**Conclusions:** The cause of death in this puppy was severe head trauma with cerebral tissue pulmonary embolism. It is believed that neural tissue gains entry into the systemic circulation through defects of the dural or meningeal venous supply by severe head trauma

#### **D-015: COR TRIATRIATUM SINISTER IN A YOUNG ADULT CAT**

Natalie Kirk, Saki Kadotani, Denae LoBato

A 2-year and 9-month-old, 4.9 kg, female spayed, Domestic Shorthair cat was presented to the University of Illinois for acute onset of respiratory distress. On presentation, the cat was tachypneic with a continuous, left parasternal, grade 3-4/6 heart murmur. A thoracic focused assessment with sonography for trauma (tFAST) scan revealed significant pleural effusion which was drained by thoracocentesis. An echocardiogram revealed a perforate membrane separating the left atrium into a proximal and distal chamber, thickened mitral leaflets, a severely enlarged right atrium and ventricle, tricuspid regurgitation, and a suspected primum atrial septal defect. Due to poor prognosis, the patient was euthanized and submitted for necropsy examination. The right atrium and pulmonary trunk were markedly enlarged and there was both concentric and eccentric hypertrophy of the right ventricle. The left atrium was divided into a proximal and distal portion by a thin membrane with a centrally located, 2 mm in diameter opening. The lumen of the left ventricle was narrowed. There were approximately 50 mL of serosanguinous pleural effusion and moderate pulmonary edema. Necropsy findings confirmed cor triatriatum sinister (CTS) with right and left sided heart failure. Histologically, there was left atrial endocardial fibrosis and marked right ventricular myocardial degeneration and fibrosis. CTS is a rare, congenital cardiac disease of debated embryologic origin that has been reported in cats, dogs, and

humans, and that is caused by separation of the left atrium into proximal and distal compartments by a thin, fibromuscular membrane.

#### **D-016: ABSENCE OF RIGHT CRANIAL VENA CAVA AND PERSISTENCE OF LEFT CRANIAL VENA CAVA IN A CALF**

Rubén López-Crespo, Mario Bedolla-Alba, Rosaura Contreras-Pérez, Johnatan Ruíz-Ramírez, Luis García-Márquez, Carlos Salas-Garrido

The aim of this report is to describe a vascular congenital anomaly in a bovine. A 3 months-old, female, Holstein bovine was submitted to the Center for Teaching and Diagnosis of Bovine Diseases of the Faculty of Veterinary Medicine and Zootecnisc of the National Autonomous University of Mexico located in Tizayuca, Hidalgo, Mexico. In the *post-mortem* study the bovine showed an anomaly of the venous system characterized by the absence of the right superior vena cava (ARSVC) and persistence of the left superior vena cava (PLSVC). This pathology originates when the involution of the left superior cardinal vein fails. PLSVC is the most common vascular anomaly in humans, and it is very rare to find the combination of ARSVC and PVCCD. Therefore, it is important to know that these anomalies also affect Bovines.

#### **D-017: ULCERATIVE VEGETATIVE AND CAVITATING BACTERIAL AORTIC VALVULAR ENDOCARDITIS IN TWO DOGS**

Tatiane Terumi Negrão Watanabe, Sarah Shippy, Fabio Del Piero

**Background:** Valvular endocarditis is common in farm animals but it is seldom observed in dogs. Cavitating endocarditis has been sporadically associated with *Bartonella* spp.

**Methods:** A 2.5-year-old, intact male, Boxer dog with a sudden death (case1) and a 4-year-old, intact male, American Bulldog with a 4-day history of anorexia, lethargy, vomiting, bloody diarrhea, and weight loss (case 2) were submitted for postmortem examination.

**Results:** In both cases, the aortic semi-lunar valves were irregular with loss of portions of the leaflets and effaced by brownish, irregular, friable, dry exudate. The endocardium behind the valves was ulcerated with significant focal loss of myocardium forming a 3.0 cm oval cavitation extending for the valve transversal length. Similar lesions affected the left atrioventricular valve (case 1). The aortic valve was replaced by neutrophils and macrophages mixed with abundant fibrin, cellular necrotic debris and edema with numerous Gram-positive cocci and Gram-negative rods and filamentous bacteria. The heart and kidney were affected by multiple infarcts and hemorrhage. No bacteria were isolated from valvular samples. PCR for detection of *Bartonella* spp. in paraffin-embedded tissue is pending.

**Conclusions:** Here we describe the findings of an unusual bacterial aortic bicuspid valvular endocarditis with associated necrosis and cavitation of the adjacent myocardium in two dogs.

#### **D-018: ATHEROSCLEROSIS IN KORAT CATS**

Veera Karkamo, Su Ngyen, Karoliina Hagner, Anna Knuuttila, Matti Jauhiainen, Henna Kairento, Niina Airas, Katariina Öörni, Ilona Kareinen

**Background:** In most companion animals, atherosclerosis is of little clinical relevance and cats are considered atheroresistant having an anti-atherogenic lipid profile. Most animal models differ from the devastating human disease either morphologically or in the lipid profile.

**Methods:** Two related adult Korat cats, a male and a female, suffered from progressive cardiovascular symptoms at different time points. The male cat died after a short period of progressive lethargy and the female cat was euthanized after a sudden onset of paraplegia. Post-mortem investigations were performed. Also blood samples were collected from 50 Korat cats and 13 controls of different cat breeds. Their serum low-density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides (TG) were measured.

**Results:** Post-mortem examinations revealed generalized thickening, hardening, loss of elasticity, and luminal narrowing of large and medium-sized arterial walls with multifocal white plaques on the inner surface in both cats. On histopathology, the arterial changes showed severe diffuse intimal thickening with accumulation of cholesterol, lipid macrophages, and mineral within the tunica intima with smooth muscle cell proliferation. The measured serum lipoprotein levels showed markedly higher values in TG and HDL in Korats.

**Conclusions:** Two Korat cats were diagnosed at autopsy with severe atherosclerosis with marked arterial intimal thickening comparable with human disease. Preliminary serum biochemistry results reveal an altered lipid profile in the studied Korat cat population compared to healthy controls. Further investigations are ongoing to discover the mechanisms behind the progressive disease and to clarify the possible role of a non-functional HDL lipoprotein pool.

#### **D-019: ANOMALOUS PULMONARY VENOUS CONNECTION IN A CALF**

Rubén López-Crespo, Mario Bedolla-Alba, Carlos Salas-Garrido, Ramón Wong-Saavedra, Lucía García-Camacho

The aim of this work is to inform a cardiac anomaly in a 14-day-old female Holstein bovine which was presented to the necropsy room of the Research Center of Bovine Disease in Tizayuca, Hidalgo. On gross examination, a defect of pulmonary veins was found as well as pulmonary edema and consolidation areas. Microscopically, arterial hypertrophy was observed in the lung. According to the gross findings and the occlusion of blood flow, the congenital defect was diagnosed as a cardiac type total pulmonary venous connection which is a rare disease that leads to secondary hypertension due to the cardiac problem. Consequently, affected animals have a poor prognosis and die of pulmonary complications such as pneumonia.

## **D-020: LOCARD'S EXCHANGE PRINCIPLE: FROM DOG TO MAN**

Adam Stern, Nicole Chinnici

**Background:** Edmund Locard described his exchange principle in the early 1900's and it essentially states that every contact leaves a trace. Locard's Principle of Exchange is now the basis for all forms of trace evidence examination. Evidence that may be left behind includes hair/fur, fingerprints, blood and other bodily fluids, which contain DNA. This same principle applies in cases of animal cruelty.

**Objective:** To describe a case of sharp force trauma in a dog in order to highlight Locard's Exchange Principle.

**Methods:** A forensic necropsy of a dog was performed and tissue samples for DNA testing were collected. Examination of the human suspect's clothes for potential bloodstains was conducted. Clothing was secured and tested for the presence of canine DNA. Using a 17 microsatellite multiplex, DNA from the canine was compared to blood stains on the suspect's clothes.

**Results:** Eighteen stab wounds were identified on the dog. Multiple tracts extend from the skin into the pleural cavity resulting in pneumothorax. Red stains were identified on the suspect's pants and boots. Reference samples from the dog and samples of the clothes matched at 17 loci. The probability of two canids having the same exact genotypic profile is 1:1,000,000,000.

**Conclusion:** Close contact as in the case of a stabbing will allow for transfer of blood spatter from victim to suspect. This transfer of blood is just one example of Locard's Exchange Principle. This case highlights the importance of trace evidence analysis to allow for formation of a linkage between suspect and victim.

## **D-021: WIDESPREAD LARGE GRANULAR LYMPHOCYTIC LYMPHOMA IN A 19-YEAR-OLD GELDING**

T. William O'Neill, Christiane Löhr, Sarah Schale, Erica McKenzie, Jennifer Johns

A 19-year-old Canadian Warmblood gelding presented with a three-week history of lethargy, inappetence, and weight loss and elevated AST (1834 U/L, RI = 175-340) and GGT (258 U/L, RI = 5-24). Pulse (64 bpm) and respiration rate (40brpm) were elevated. Ultrasound revealed hepatomegaly and heterogeneity of the spleen. Complete blood count showed lymphopenia with rare large granular lymphocytes. Abdominal fluid cytology identified a population of large granular lymphocytes (LGL) consistent with LGL lymphoma. At necropsy, the spleen, liver, lung, and kidneys contained variably sized, firm, tan, discrete masses. There was diffuse lymphadenomegaly in the abdomen and thorax with the largest lymph node measuring 5cm by 3cm. Cytologic impressions of viscera and lymphatic tissues confirmed the LGL lymphoma. Histopathologically, lymph node architecture was effaced by large lymphocytes that rarely had visible cytoplasmic granules. Infiltrative lymphocytes accompanied by scattered eosinophils were identified in the liver, lung, and spleen and were associated with expansive fibrosis in the splenic capsule and lungs. The intestinal mucosa was expanded by small lymphocytes with

fewer eosinophils, interpreted as enteritis. Immunohistochemistry for CD3 on spleen, lymph node, and lung strongly stained neoplastic lymphocytes confirming T cell origin, consistent with previously reported cases of LGL lymphoma in horses. Lymphoma of LGL is rare in horses and can become widely disseminated. This case exemplifies effective communication between clinicians, clinical pathologists, and anatomic pathologists.

#### **D-022: GRANULOCYTIC SARCOMA IN A DOG WITH MYELOID LEUKEMIA**

Miguel Madrigal-Alvarado, Alonso Reyes-Matute, Mónica Samperio-Gama, Ana Carvajal-Rincon

Granulocytic sarcoma or chloroma, named for its green appearance, is a rare manifestation of a myeloproliferative disorder. It is defined as an extramedullary mass composed of immature myeloid cells. A 2.5-year-old, male, Welsh corgi dog diagnosed with chronic granulocytic leukemia by bone marrow aspiration was presented for postmortem examination. On gross examination a pale green material was found completely replacing the cortex of both kidneys, the parenchyma of several lymph nodes, the epicardium, the pulmonary pleurae, the hepatic capsule and also inside the lateral ventricles of the brain. Microscopically, this green material was composed of neoplastic granulocytes with strong intracytoplasmatic immunopositivity to myeloperoxidase and lysozyme. Electron microscopy was performed where several intracytoplasmic granules were also identified. These findings are consistent with a granulocytic sarcoma which is a rare neoplasm uncommonly diagnosed in domestic animals.

#### **D-023: EVALUATING THE EFFECTS OF VARIOUS DECALCIFICATION PROTOCOLS ON IMMUNOHISTOCHEMICAL STAINING IN ZEBRAFISH (DANIO RERIO)**

Danielle Meritet, Christiane Loehr, Sean Spagnoli, Kay Fischer

**Background:** Both fixation and decalcification can alter protein structure in tissues and may impact the efficacy of primary antibodies routinely used in immunohistochemistry.

**Objective:** We investigated the effect of commonly applied fixation and decalcification protocols on immunohistochemical staining in zebrafish.

**Methods:** Zebrafish were fixed for varying times in Dietrich's fixative or 3.4% neutral buffered formaldehyde followed by decalcification with EDTA. Three independent observers evaluated sections stained for cytokeratins, vimentin, GFAP, S100, chromogranin A, and muscle actin by immunohistochemistry.

**Results:** Overall, interobserver variation was moderately high. The type of fixative had different effects for individual markers, reducing or increasing staining intensity; a negative time-dependent effect was detected for several antibodies. Of note, the use of Dietrich's fixative greatly reduced the staining intensity for the neural markers: GFAP, Chromogranin A and S100. The decalcification protocols yielded unexpected results, with longer durations leading to more intense staining scores for one marker.

**Conclusions:** No single fixation protocol worked best for all antibodies. We do recommend NBF/CalExII for immunohistochemical studies of neural tissue in zebrafish.

#### **D-024: IMMUNOHISTOCHEMICAL DETECTION OF VIRAL ANTIGENS FROM DIFFERENT MORBILLIVIRUS-INFECTED SPECIES**

Giovanni Di Guardo, Daniela Malatesta, Stefania Salucci, Ludovica Di Renzo, Guendalina Zaccaria, Alessio Lorusso, Eliana De Luca, Anna Rita D'Angelo, Federica Monaco, Margherita Piserchia, Pasquale Troiano, Antonio Petrella, Gabriella Di Francesco

The genus *Morbillivirus* of the *Paramyxoviridae* family encompasses lympho-epithelio-neurotropic RNA viruses infecting several species, including aquatic mammals. The present study was aimed at evaluating the immunohistochemical (IHC) reactivity of tissues from different species to 3 anti-morbilliviral antibodies (Abs). The brain and lung of *Morbillivirus*-infected wolves (*Canis lupus*), badgers (*Meles meles*), foxes (*Vulpes vulpes*), dogs (*Canis familiaris*), and striped dolphins (*Stenella coeruleoalba*), along with the kidney of *Morbillivirus*-infected badgers, kidney, and urinary bladder from *Feline Morbillivirus* (FeMV)-infected cats (*Felis catus*), were investigated by means of IHC and quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The aforementioned Abs identified morbilliviral antigens in all tissues from the 6 species under study, with the only exception of the anti-*Canine Distemper Virus* (CDV) Ab, which yielded negative results on kidneys and urinary bladders from FeMV-infected cats. The positive IHC reactions observed in tissues from the herein investigated species were the likely result of the antigenic cross-reactivity among the viruses infecting them. In this respect, being the anti-FeMV and the anti-CDV a polyclonal and a monoclonal Ab, respectively, the reaction's sensitivity was presumably enhanced by the anti-FeMV Ab, as in the case of the anti-PPRV Ab, while the reaction's specificity was increased by the anti-CDV Ab.

In conclusion, investigations of this kind may enhance our diagnostic capability and knowledge about the pathogenesis of *Morbillivirus* infections.

#### **D-025: HISTOPATHOLOGY AND ELECTRON MICROSCOPY OF CHLAMYDIA PNEUMONIAE ENCEPHALOMYELITIS AND GANGLIONITIS IN CAPTIVE HOUSTON TOADS (ANAXYRUS HOUSTONENSIS)**

Alycia Fratzke, Lauren Howard, Maryanne Tociłowski, Anibal Armien, Fabiano Oliveira, Branson Ritchie, Erin Berlin, Eric Snook

**Background:** *Chlamydia pneumoniae* is an ubiquitous, gram negative, intracellular bacterium causing disease in humans, mammals, birds, reptiles, and amphibians. Since 2012, *C. pneumoniae* infection has caused neurologic disease and mortality in a breeding colony of endangered Houston toads (*Anaxyrus houstonensis*) at the Houston Zoo. Definitive diagnosis of *C. pneumoniae* in these animals requires histopathologic evaluation, however the histopathologic features of this disease have not been previously reported.

**Objective:** The purpose of this report is to characterize the histopathologic and electron microscopic features of *C. pneumoniae* infection in the Houston toad.

**Methods:** Histopathology slides from previously submitted cases of confirmed or suspected Chlamydiosis were systemically evaluated for characteristic lesions. In one case, tissues were evaluated using both histopathology and electron microscopy.

**Results:** The major histopathologic finding is a necrotizing and histiocytic encephalomyelitis and ganglionitis. Bacteria form intracytoplasmic inclusions within neurons but frequently spill into the surrounding tissue from necrotic cells. Additionally, one case revealed granulomatous inflammation in the liver with intrahepatocytic bacterial inclusions. Ultrastructural evaluation shows the bacteria form reticulate and elementary bodies characteristic of *Chlamydia* spp.

**Conclusion:** This is the first report of natural *C. pneumoniae* infection causing encephalomyelitis in a veterinary species. Although the source of the bacteria and point of entry are unknown, the presence of *C. pneumoniae* in the liver suggests a potential hematogenous route of infection.

#### **D-026: SPOROTRICHOSIS IN CATS FROM SOUTHEAST BRAZIL**

Tamires Teodoro, Fernanda Souza, Laice Silva, Maria Campos, Mary Varaschin, Flademir Wouters, Djeison Raymundo, Angelica Wouters

**Background:** Sporotrichosis is a worldwide mycosis, caused by *Sporothrix* sp. This organism is a dimorphic fungus, growing as hyphae at environmental temperatures and in yeast form in tissues. It is a chronic infection usually limited to the skin and subcutaneous tissue and causes disease in humans, cats, and other species. Transmission occurs through the skin by traumatic inoculation with contaminated soil, plants, and organic matter. Cytologic and histologic exams are important tools for the diagnosis of the disease.

**Objective:** This report describes the results of necropsies, histopathologic and cytopathologic exams of 21 cases of sporotrichosis in cats.

**Methods:** All samples collected at necropsy or received for histopathology were processed on histologic routine techniques and cytologic samples were stained with a Romanowsky-type stain.

**Results:** Gross lesions were found on the skin of the head, neck and limbs, which consisted of alopecia, exudative or crusted confluent ulcerations, and some animals had also enlarged lymph nodes and/or lesions in the nasal cavity. Histologic findings consisted mainly of pyogranulomatous and ulcerative dermatitis containing macrophages filled with numerous yeast-like forms of *Sporothrix* sp., also found in lymph nodes in one cat. The yeasts were round, oval, or elongated.

**Conclusion:** The diagnosis of sporotrichosis outbreaks has been increasing in the Southeast of Brazil. Due to the zoonotic character of this disease, it is necessary to improve the knowledge about the morphologic lesions involved in this illness, since the

transmission from cats to humans has been associated with the high number of yeasts in lesions.

#### **D-027: ALVEOLAR ECHINOCOCCOSIS IN A PET DOG IN MISSOURI**

Keiichi Kuroki, Yasuyuki Morishima, Lindsay Dorr

A 10-year-old, male neutered Boxer that was born and lived in Missouri, developed intermittent vomiting and diarrhea and became anorexic over the course of several months before passing away. A postmortem examination revealed the liver was largely replaced by a pale tan mass with surface irregularity. Microscopic findings of the liver lesion were compatible with alveolar hydatid disease caused by *Echinococcus multilocularis*. Diagnosis of alveolar echinococcosis was confirmed by PCR testing. Mitochondrial DNA haplotyping is in progress. This is the first canine alveolar echinococcosis case and the second pet dog that was confirmed to be infected by *E. multilocularis* in the contiguous United States. *E. multilocularis* can be a serious health risk for both pet dogs and humans. The present case reminds veterinary pathologists and animal and human health professionals that *E. multilocularis* may have been established in a wider region of the contiguous United States.

#### **D-028: CYTAUXZONOSIS IN A YOUNG DOMESTIC SHORTHAIRED CAT**

Christopher Lanier, I. Dan Kuykendall II, Laura Lowe, Lenore Bacek, Joseph Newton

**Background:** An 11-month-old spayed female domestic shorthaired cat was referred to the Auburn University Veterinary Teaching Hospital for fever, lethargy, and severe icterus. The cat was weak with a “stumbling” gait and vomited yellow liquid (one episode reported) on the morning of presentation. A CBC demonstrated moderate to marked non-regenerative anemia, moderate leukopenia characterized by severe neutropenia with a marked left shift, and severe thrombocytopenia. Serum biochemistry revealed marked hyperbilirubinemia (11.13 mg/dL; reference interval (RI) = 0.10-0.20 mg/dL) and mild ALT (95, RI = 26-77 U/L) and AST (144 U/L, RI = 12-45 U/L) increases. The cat was indoor-outdoor and up to date on immunizations.

**Objective:** To demonstrate clinicopathologic, cytologic, and histopathologic findings associated with this disease syndrome.

**Methods:** A peripheral blood smear, lymph node fine needle aspirates (FNAs), and histopathologic sections of multiple tissues (to include lungs, liver, spleen, and lymph nodes) were examined by light microscopy.

**Results:** Peripheral blood smear review revealed numerous characteristic 1.0-2.0 µm intraerythrocytic piroplasm-like merozoites in addition to several large protozoal schizonts within mononuclear cells at the feathered edge. Cytologic and histopathologic lymph node evaluation revealed many similar mononuclear cells markedly expanded by protozoal schizonts. Many similar schizonts were observed within vasculature of virtually all tissue sections examined microscopically.

**Conclusions:** This report describes a classic presentation of *Cytauxzoon felis* with hematologic, cytologic, and histopathologic evidence of various life cycle stages.

#### **D-029: SYSTEMIC ARTERITIS ASSOCIATED WITH PCV3 INFECTION IN POST-WEANING PIGS**

Timothy Carlson, Talita Resende, Fabio Vannucci

Tissues were submitted from multiple 5 to 7-week-old, post-weaning pigs with inappetance and weight loss. Gross necropsy, performed on the farm, reported no lesions. Histopathologic examination showed systemic lymphoplasmacytic and histiocytic arteritis most remarkably observed in the myocardium, with interfascicular edema and infiltration of mononuclear cells in the walls of blood vessels, often with focal hyperplasia of the tunica intima. PCR for porcine circovirus 3 (PCV3) was positive with Ct values of 22-23. In situ hybridization (ISH) for the viral mRNA targeting ORF2 gene (open reading frame 2, encoding the viral capsid) showed colocalization of the probe to the lesions. These findings are further evidence for the role of PCV3 as a cause of arteritis, which is currently a sporadic but important differential diagnosis as a cause for such lesions. Differentiation of PCV3 and PCV2 associated lesions has important management implications as there is no commercial vaccine for PCV3 and this agent continues to be detected across the globe. Furthermore, this serves as continued evidence for the importance of surveillance and characterization of pathogenesis for this agent.

#### **D-030: INTRANUCLEAR COCCIDIOSIS IN A TWO LEOPARD TORTOISES**

Molly Friedemann, Laura Rice, Brooke Griff, J Heatley, Angela Arenas, Raquel Rech

An adult female leopard tortoise (*Stigmochelys pardalis*) presented to Texas A&M University Veterinary Medical Teaching Hospital (VMTH) with a two-week history of egg deformation, increased egg-laying time, anuria, and acute foam production from the nose and mouth. The patient continued to decline with worsening respiratory distress and humane euthanasia was elected. Postmortem examination revealed a marked acute gastroenterocolitis with severe edema and congestion, and hepatic necrosis. Histology showed a marked gastroenterocolitis and hepatitis with abundant intranuclear coccidia in mucosal epithelial cells and hepatocytes. Significant renal tubular necrosis with rare tubular epithelial intranuclear coccidia and splenic necrosis were also identified. One month later, an adult male leopard tortoise from the same collection presented to the VMTH with a two-week history of lethargy and anorexia. Oral and conjunctival swabs taken for an antemortem intranuclear coccidiosis PCR were positive, and despite supportive care and anticoccidial administration, the animal continued to deteriorate and died. Significant gross and histologic changes included a marked chronic gastritis, splenitis, pancreatitis, and nephritis, as well as a more acute rhinitis and glossitis. No coccidia were identified via light microscopy. Infections with intranuclear coccidiosis of tortoises (TINC) are often systemic and cause nonspecific clinical signs and significant morbidity and mortality in affected animals. There is still much to be discovered regarding TINC transmission, route of infection, pathogenicity, and the clinical implications of infection. These cases represent an interesting small outbreak within a private collection and highlight the range of lesions that may be seen with TINC.

### **D-031: PENTASTOMIASIS IN CAPTIVE JUVENILE AMERICAN ALLIGATORS**

Erin Graham, Jacob Steventon, Jennifer Wawra, Christian Keller, April Childress, James Wellehan, Heather Walden, Salvatore Frasca, Jr., Robert Ossiboff

Two 2-year-old, captive bred, American alligators (*Alligator mississippiensis*) housed in a mixed exhibit in a public aquarium spontaneously died following a short period of anorexia, lethargy, and oral hemorrhage. The most significant findings in both individuals were severe pulmonary hemorrhage with arteritis and thrombosis. High numbers of pentastome nymphs were present within vessels and the airways. Nymphs recovered from the lung were morphologically consistent with *Sebekia mississippiensis*. Additional findings included segmental, necrotizing enteritis and high numbers of pentastome nymphs within systemic vessels. Death was attributed to pulmonary hemorrhage secondary to severe pentastome nymph migration. To determine if the alligators acquired the parasites from their exhibit, postmortem examinations were performed on cohabitant fish. High numbers of pentastome nymphs were identified in mosquito fish (*Gambusia affinis*), golden shiners (*Notemigonus crysoleucas*), and green sunfish (*Lepomis cyanellus*). Nymphs recovered from golden shiners and green sunfish were also morphologically consistent with *S. mississippiensis*. A region of the small subunit ribosomal RNA gene was amplified by PCR from nymphs found in the alligators and fish. Sequencing revealed 100% identity to each other as well as previously published *Sebekia* spp. The cases in this report document the array of lesions associated with *Sebekia* nymph migration in American alligators. Pentastomiasis is an important disease in captive juvenile crocodilians for which there are limited available treatment options. Furthermore, while mosquito fish have been previously reported as an intermediate host for *Sebekia mississippiensis*, this is the first report of the parasite in golden shiners and green sunfish.

### **D-032: AN OUTBREAK OF SARCOCYSTOSIS AND SALMONELLOSIS IN A GROUP OF PSITTACINES**

Ryan Taylor, Alicia Olivier

**Abstract:** Three psittacines from a zoological park were necropsied at Mississippi State University College of Veterinary Medicine. Eight birds were reported to have died out of a census of approximately 150 with no recent introductions. Of those necropsied, one had diarrhea and weight loss with no reported clinical signs in the other two birds. Histological findings included interstitial pneumonia, pulmonary edema, hepatocellular necrosis, splenitis and enteritis. Within the lungs were intra-endothelial protozoal schizonts consistent with *Sarcocystis falcutula*. Aerobic culture of the small intestines and liver from all three birds was positive for *Salmonella*, identified as *S. typhimurium* via serology. The immediate cause of death was pulmonary edema associated with Sarcocystosis. The hepatocellular necrosis, splenitis and enteritis were secondary to systemic Salmonellosis. Psittacines are an intermediate host for the protozoa, with opossums serving as the definitive host. The cockroach is a common vector, feeding on opossum feces and harboring sporocysts, in addition to serving as a potential vector for *Salmonella*.

### **D-033: RHODOTORULA MUCILAGINOSA ASSOCIATED WITH PULMONARY ADENOCARCINOMA IN A CAT**

Melissa Roy, Kevin Woolard

*Rhodotorula* species are saprophytic yeasts that are an emerging pathogen, primarily in immunocompromised patients. Opportunistic infections of skin, meninges, eyes, peritoneum, and lungs have been reported in humans.

We present a case of an 11-year-old, female, spayed domestic shorthair cat that developed progressive respiratory distress over the course of several months. Thoracic radiographs revealed coalescing nodular opacities throughout all lung lobes overlying a diffuse miliary pattern. Bronchioalveolar lavage had suppurative inflammation with marked epithelial cell atypia. Cultures of this fluid were uninformative. The patient was treated with prednisolone and diagnosed with pulmonary carcinoma. Approximately one month later, the patient re-presented with progression of clinical signs, was euthanized, and submitted for necropsy.

On gross examination, approximately 80 percent of the lungs had variably sized, well-demarcated, pale tan, firm regions of parenchyma. Histologically, lung contained well-demarcated regions of pulmonary carcinoma in which up to 25 percent of the neoplastic cells contained intra-cytoplasmic, variably sized, yeast organisms. The organisms stained positive with Grocott's methanamine silver (GMS). A section of fresh, frozen (-20 degrees C) lung tissue was submitted for fungal culture, which grew very small numbers of pigmented yeast, consistent with *Rhodotorula mucilaginosa* (by MALDI-TOF). Presence of *Rhodotorula mucilaginosa* was confirmed in lung tissue using PCR with ITS1/ITS2 and ITS3/4 primer sets.

This case outlines an unusual presentation of an opportunistic infection with a common environmental fungus.

### **D-034: CASE REPORT ASSOCIATED WITH ASPERGILLOSIS AND HEPATITIS E VIRUS COINFECTION IN HIMALAYAN GRIFFONS**

Tianlong Liu, Ruiping She, Jijing Tian, Junqing An, Chenglin Zhang

**Background:** This study describes the deaths of four Himalayan griffons housed in the Beijing Zoo. Based on pathogen identification and the pathological changes observed, we characterized a mixed infection of fungi and Hepatitis E virus (HEV) in all four birds.

**Results:** Pathological changes were severe. Membranous-like material was observed on the surface of the internal organs. The spleen was necrotic. Focal lymphocytic infiltration of the liver was observed and there were many sunflower-like fungal nodules in multiple tissues, especially in the kidney. PCR was used to identify the pathogen and, based upon the 18S-rRNA genomic sequences of known fungi, results confirmed that all four dead Himalayan griffons were infected with *Aspergillus*. At the same time, PCR for HEV was also positive.

**Conclusion:** To the best of our knowledge, this work appears to be the first report of the concurrent detection of *Aspergillosis* and Hepatitis E virus in a rare avian species.

### **D-035: GENOTYPE IV HEPATITIS E VIRUS CROSS SPECIES INFECTION IN CAPTIVE SIBERIAN TIGER BY FOOD BORNE TRANSMISSION**

Ruiping She, Tianlong Liu, Jijing Tian, Chenglin Zhang

**Background:** Accumulated evidence has demonstrated that hepatitis E (HE) is a zoonotic disease and various animal species have serum antibodies to HEV. Recently, much evidence suggests that individuals closely working with swine are at high risk of HEV infection. However, little is known about HEV infection in captive wild animals.

**Methods:** In March 9, 2016, one Siberian tiger died from a malignant tumor in the Beijing Zoo. Major tissues, fresh fecal samples, and 20 food samples were collected and examined by RT-nPCR and standard histopathological analyses.

**Results:** HEV RNA was detected in the liver, small intestine and large intestine samples. Testing of fecal samples from the other four Siberian tigers at the zoo revealed that one was also positive. PCR results also showed that one food sample (beef liver) was positive. Sequences were blasted in Genbank and showed that the detected virus belongs to genotype 4 and was closely related to other tiger HEV (99% identity). The histological lesion of the liver showed periportal infiltration of inflammatory cells and fibrosis suggesting chronic hepatitis. With immunohistochemical staining, HEV antigen was detected in liver and intestine.

**Conclusion:** The present data first report genotype IV HEV cross-species infection in Siberian tigers. Detection of genotype IV HEV in the beef liver food sample of these Siberian tigers suggests that food-borne transmission can be hypothesized as the way of infection.

### **D-036: ENCEPHALITOZOON POGONAE-ASSOCIATED VASCULITIS IN AN INLAND BEARDED DRAGON (POGONA VITTICEPS)**

Nicole Kaiser, Spencer Greenwood, Shannon Martinson, Gillian Gouchie

**Introduction:** A new species of microsporidia, *Encephalitozoon pogonae*, is an emerging cause of systemic granulomatous disease in Inland Bearded Dragons. We herein describe the case of a four-year-old female inland bearded dragon that presented with a 5-6 day history of straining to defecate due to the presence of a 2x1 cm coelomic mass located adjacent to the left hindlimb. Surgery was performed to debulk the leg mass; post-surgically she continued to decline and died 2 months after initial presentation and was submitted for necropsy examination.

**Objective:** To describe novel vascular lesions associated with *Encephalitozoon pogonae* in an Inland bearded dragon.

**Results:** Necropsy examination revealed granulomatous dermatitis, myositis/cellulitis, coelomitis, encephalitis, pneumonia, and multiple mass-type lesions. One mass was present in the pelvic canal preventing defecation and two were within the pharynx and appeared grossly to be hematomas. Prominent vasculitis with intralesional microsporidia was detected in multiple organs and, in areas, resulted in aneurysmal dilation and perivascular hemorrhage. A diagnosis of systemic microsporidiosis with

vasculitis was made. DNA extraction, PCR and sequencing were completed on five independent tissue samples, and revealed 100% match with *Encephalitozoon pogonae* across the 132bp shared by the following sequences: JQ682643, KR998311, and LC214885.

**Conclusion:** To our knowledge, this is the first reported case of vasculitis caused by *E. pogonae* in an inland bearded dragon.

#### **D-037: RENAL DISEASE IN TWO REPTILES WITH FLAGELLATED PROTOZOA CONSISTENT WITH HEXAMITA PARVA**

Allison Watson, Shelley Newman, Andrew Cushing, Linden Craig

Flagellated protozoa are common in the gastrointestinal tract of reptiles where they are presumed to be nonpathogenic. Renal disease caused by the diplomonad flagellated protozoan *Hexamita parva*, has been previously described in chelonians and chameleons, and rarely in other lizard and snake species. Infection occurs through ingestion, and flagellates are thought to reach the urogenital tract by ascending from the cloaca. Here we describe cases from a veiled chameleon (*Chamaeleo calypttratus*) and a bog turtle (*Glyptemys muhlenbergii*) with renal disease and flagellated protozoa in the kidney and small intestine, respectively. Renal lesions included mild renal tubular degeneration with rare urate tophi (chameleon) and chronic active necrotizing and heterophilic tubulointerstitial nephritis (bog turtle). Many 2x3 micrometer piriform flagellated protozoa with two apical nuclei, morphologically consistent with *Hexamita parva*, were within ectatic renal tubules in the chameleon kidney, and within the small intestinal lumen of the bog turtle, associated with mild mucosal loss. Urine, obtained via cystocentesis, was examined from the bog turtle's surviving conspecific and motile flagellated protozoa were present, supporting the diagnosis of *Hexamita parva*. The chameleon had extensive mineralization of dermal collagen and vascular smooth muscle in multiple organs. Regionally extensive necroulcerative dermatitis with rare dermal mineralization was present in the bog turtle. Metastatic mineralization caused by renal secondary hyperparathyroidism may have contributed to soft tissue mineralization in these cases. Although usually nonpathogenic in the gastrointestinal tract, *Hexamita parva* should be considered as a cause of renal disease in reptiles with renal or intestinal flagellated protozoa.

#### **D-038: NEOSPOROSIS IN A 3-MONTH-OLD CALF WITH HINDLIMB PARALYSIS**

Rory Chien, Melanie Breshears, Anthony Confer

A 3-month-old, female, Aberdeen-Angus calf (*Bos taurus*) was necropsied. This calf had an approximately 1-week history of paraparesis and inability to stand that progressed to lateral recumbency and hindlimb paralysis prior to euthanasia. At necropsy, multifocal skeletal myonecrosis was seen throughout the body and prominent multifocal myocardial necrosis was also noted. A regionally extensive focus of myelomalacia was observed in the lumbar spinal cord. Histopathology revealed severe necrotizing myocarditis, myositis and variable degrees of meningoencephalomyelitis throughout the CNS with necrosis, lymphohistiocytic to mixed inflammatory infiltrates, and numerous intralésional protozoal tachyzoites and tissue cysts, compatible with bovine

neosporosis. Ultrastructurally, the protozoa contained many electron-dense rhoptries and numerous micronemes, consistent with *Neospora caninum*. Neosporosis is a significant cause of abortion and stillbirth in cattle worldwide. Most of the calves and adult cattle are subclinically infected, but uncommonly, diseases related to *N. caninum* infection, especially neurologic deficits, can be observed in young calves less than 4 months of age. Here we present a case of neosporosis in a calf with hindlimb paralysis and disseminated myonecrosis.

#### **D-039: PATHOLOGY OF PNEUMONIC PLAGUE IN A DOG**

Lauren Harris, Josh Daniels, Stephanie Brault, Connor Hershkowitz, Paul Morley, Kristy Dowers, Paula Schaffer

**Background:** *Yersinia pestis* is a reportable zoonosis and the causative agent of plague. Cases of plague occur in humans and animals in the western United States, usually during spring through fall months. Dogs typically present with either the septicemic or bubonic form. Pneumonic plague is rare in dogs.

**Objective:** We describe the unique clinical and diagnostic features of pneumonic plague in a dog.

**Methods:** Routine histopathology and gram stain were performed on surgical and post-mortem tissues. MALDI-TOF, Vitek2, and *Yersinia*-specific PCR were applied to bacteria isolated from lung.

**Results:** A mixed breed dog presented to the Colorado State University Veterinary Teaching Hospital in December for evaluation of lethargy, fever, and hemoptysis. Thoracic radiographs and CT demonstrated right accessory lung lobe consolidation. The dog was treated by lobectomy. Histopathology revealed severe hemorrhagic and necrosuppurative pneumonia without bacteria, presumably due to antimicrobial therapy. An isolate cultured from a surgical swab was misidentified as *Yersinia pseudotuberculosis* (by MALDI-TOF) and *Pseudomonas luteola* (by Vitek2). PCR was diagnostic for *Yersinia pestis*. Post mortem, pathology was diffuse in all lung lobes and there was necrotizing tonsillitis.

**Conclusions:** Atypical features of this case included presentation in winter, initial lobar pattern, lack of bacterial colonies in histologic sections, and bacterial misidentification by automated systems. These features delayed the rapid diagnosis necessary for effective public health response. Efficient identification of this virulent zoonotic agent may be facilitated by consideration of pneumonic plague in dogs presenting with fever and hemoptysis and with possible vector exposure regardless of season.

#### **D-040: NASAL MYCOBACTERIOSIS IN A HORSE**

Krystal Vail, Lauren Stranahan, Lauren Richardson, Dominique Wiener, Brian Porter

A 22-year-old Quarter Horse gelding presented for a 2 month history of chronic mucopurulent discharge from the left nares. Upper airway endoscopy and computed tomography revealed an expansile mass occluding the left paranasal sinus with

destruction of the ethmoid turbinates, effacement of the left dorsal and ventral conchae, and deviation of the nasal septum. Due to the extent of the mass, the age of the horse, and concurrent chronic hind limb lameness, euthanasia was elected. Grossly, abundant purulent material exuded from the left nostril. On sagittal sectioning of the head, a 16x8x4 cm, soft, yellow mass with purulent exudate occluded the mid to caudal left nasal cavity and the left rostral and caudal maxillary and sphenopalatine sinuses, eroding the nasal conchae and sinus mucosa. Histologically, the mass consisted of numerous epithelioid macrophages, multinucleated giant cells, and fewer lymphocytes and plasma cells with multifocal areas of necrosis and degenerate neutrophils. An acid fast stain revealed numerous acid fast bacilli within macrophages and giant cells. Aerobic culture did not yield acid fast organisms. Thus DNA was extracted from paraffin embedded tissue and universal *Mycobacterium* primers were used to amplify a portion of the 16S-23S ribosomal internal transcribed spacer region. Sequencing of the PCR amplicon matched *Mycobacterium avium intracellulare* with 98% sequence identity in GenBank. Mycobacteriosis has been infrequently reported in horses and is thought to be acquired via ingestion, as most cases are associated with granulomatous alimentary lesions. This report describes an unusual case of mycobacteriosis presenting as a nasal mass.

#### **D-041: DESCRIPTION OF AN OUTBREAK OF FRANCISELOSIS (*FRANCISELLA NOATUNENSIS* SUBSP. *ORIENTALIS*) IN NEON JEWEL FISH (*HEMICHROMIS BIMACULATUS*) FROM A FARM OF THE STATE OF MORELOS, MEXICO**

Rubén López-Crespo, Alonso Reyes-Matute, Laura Romero-Romero, Luary Martínez-Chavarría, Luis García-Márquez, Alheli Lugo-García

The aim of this report is to describe an outbreak of Francisellosis (*Francisella noatunensis* subsp. *orientalis*) in neon jewel fish (*Hemichromis bimaculatus*) from a farm in the state of Morelos, Mexico. In December 2017, 10 neon jewel fish (*H. bimaculatus*) were submitted to the Department of Pathology of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico. They came from a farm located in Morelos, Mexico. Gross examination, fresh analysis, cytology, histopathology and PCR were performed. The fish showed lethargy, erratic swimming, imbalance and gasping. On necropsy, multiple granulomas were observed in the anterior kidney, posterior kidney and spleen. Microscopically, granulomatous inflammation was observed in several organs. The presence of *Francisella noatunensis* subsp. *orientalis* was demonstrated by PCR using specific primers and DNA extracted from paraffin-embedded tissues from all the affected fish. This is the first report of Francisellosis in the neon jewel fish (*H. bimaculatus*) and the first report of the disease in an ornamental species in Mexico.

#### **D-042: MULTI-SYSTEMIC SARCOCYSTIS NEURONA INFECTION IN A BLOODHOUND**

Kathleen Mulka, Lisa Mangus

*Sarcocystis neurona* is the organism most commonly associated with equine protozoal myeloencephalitis (EPM), a serious neurological disease in horses. However, *S. neurona* can also cause disease in a variety of different species including cats, skunks,

raccoons, horses, dogs, and marine mammals. There are limited reports of *S. neurona*-like infections in dogs. This case report illustrates an example of a multi-systemic *S. neurona* infection in a 22-month-old intact male bloodhound on long-term cyclosporine therapy for atopic dermatitis. Clinical signs of *S. neurona* infection included rapid weight loss, progressive inappetence, and acute-onset fever. Partial gross necropsy revealed generalized ecchymosis and icterus, severe hepatomegaly, and dozens of firm, pale-yellow nodules throughout the lungs and liver. Microscopically, there was severe necrotizing pneumonia and hepatitis with abundant intralesional apicomplexan organisms that showed intense positive immunostaining for *S. neurona* and no positive reactivity for *Toxoplasma gondii* or *Neospora caninum*. PCR on formalin-fixed paraffin-embedded tissue was negative for *Neospora spp.* and positive for *Sarcocystis spp.* Subsequent sequence analysis of the *Sarcocystis* PCR product showed 100% identity to *S. neurona*. This case provides a rare example of *S. neurona* infection in a canine, and demonstrates that *S. neurona* should be considered as a differential diagnosis for necrotizing hepatitis and pneumonia in dogs, especially in instances of immunosuppression.

#### **D-043: CHRONIC IDIOPATHIC CHOLANGIOFIBROSIS IN TWO PET GUINEA PIGS**

Elizabeth Alloway, John Cullen

We describe two cases of chronic idiopathic cholangiofibrosis in pet guinea pigs. Both guinea pigs (ages 3.5 and 6.5 years) had a history of lethargy and anorexia of approximately 1-2 weeks duration. Limited ante-mortem diagnostics were performed due to poor prognosis. At necropsy, both guinea pigs had diffusely yellow livers that were tough and resisted crushing. Moderate to severe gall bladder distension was observed in both animals; additionally, ascites and generalized icterus were noted in one guinea pig. Histologically, both animals exhibited moderate hepatocellular lipid vacuolation and centrilobular to massive hepatic necrosis with replacement by fibrosis and ductular reaction with minimal lymphocytic infiltrates. In the guinea pig with generalized icterus, small to large bile ducts frequently contained intraluminal bile aggregates. The histologic features of guinea pig chronic cholangiofibrosis have been described in a single case report of Jena: Alb outbred guinea pigs: in these guinea pigs, necropsy revealed coagulative hepatocyte necrosis with replacement by bridging fibrosis, ductular reaction, and mild lymphocytic infiltrates. While gall bladder distension was frequently observed, obstructive biliary disease was not reported. The pathogenesis of this histologic lesion is unclear: an infectious agent was not identified and a transient toxic exposure was suspected. An underlying cause for these histologic findings was not identified in either of our cases, with no infectious organisms or history of toxicity identified. These lesions may represent the sequelae of a chronic, functional cholestasis without an observed mechanical obstruction. This case report describes the first cases of chronic cholangiofibrosis in pet guinea pigs.

#### **D-044: NESIDIOBLASTOSIS IN THREE BABOONS (PAPIO SPP.)**

Emily Corbin, Edward Dick Jr., Olga Gonzalez, Shyamesh Kumar

**Background:** Nesidioblastosis, etymologically indicating “islet proliferation”, is defined as a non-neoplastic proliferation of pancreatic islet and ductular tissue of undetermined

pathogenesis. We report nesidioblastosis in three adult baboons (*Papio* spp.). Nesidioblastosis has been reported in several veterinary species including non-human primates, but to the authors' knowledge this is the first report in baboons.

**Methods:** Three colony cull experimentally non-naïve baboons (a 17-year-old female, 19-year-old male, and 19-year-old female) were euthanized, all tissues were processed conventionally, and histology was reviewed. Congo red histochemical staining and immunohistochemical staining for AE1/AE3, CK19, synaptophysin, chromogranin, insulin, and glucagon were performed on serial sections of pancreas.

**Results:** Histologically, pancreases contained multifocal to diffuse, well-defined proliferations of co-mingled islets and ductular structures. Immunohistochemistry revealed a mixture of epithelial ductular cells (immunoreactive for AE1/AE3 and CK19) and neuroendocrine cells (immunoreactive for synaptophysin and chromogranin) composed of both  $\alpha$  and  $\beta$  islet cells (immunoreactive for glucagon and insulin). Islet amyloid was present in two of the three baboons and confirmed with congo red stain. One of the two baboons with islet amyloidosis had concurrent mild to moderate hyperglycemia.

**Conclusions:** We describe pancreatic islet and ductular proliferation in three baboons (*Papio* spp.) resembling nesidioblastosis in humans. Histopathologic findings were interpreted to be non-protocol related. This may represent a regenerative response to an unknown pancreatic insult or may represent a response to  $\beta$  cell trophic factors such as occurs in type II diabetes, for which baboons are a well-known model.

#### **D-045: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF GALL BLADDER MESENCHYMAL TUMORS IN DOGS**

Amy Flis, John Cullen

**Background:** The gall bladder is an uncommon site for mesenchymal tumors in domestic animals, and in the dog benign gall bladder mesenchymal tumors are rarely reported and typically diagnosed as leiomyomas. In the gastrointestinal tract, it is now recognized that suspected leiomyomas require further classification because gastrointestinal stromal tumors (GIST) and leiomyomas are often histologically indistinguishable. Immunohistochemical stains are the diagnostic tool of choice for differentiating the two neoplasms. In addition to the gold standard immunohistochemical marker KIT (CD117), recently DOG1 (discovered on gastrointestinal stromal tumors protein 1) has been identified as a specific and sensitive marker for canine GISTs.

**Objective:** This study describes the histologic features of a small cohort of mesenchymal tumors in the gall bladder of dogs and further characterizes them using immunohistochemical markers to distinguish between leiomyomas and GISTs.

**Methods:** Six mesenchymal tumors of the gall bladder in dogs were identified in the NC State University archives from January 2010 to May 2018, and, in addition to H&E evaluation, immunohistochemical markers for smooth muscle actin (SMA), KIT (CD117), and DOG1 were applied to sections of the tumors.

**Results:** Preliminary results suggest that one case is a GIST based on positive immunoreactivity for DOG1, and the other five neoplasms are leiomyomas. All have positive immunoreactivity for SMA.

**Conclusions:** Mesenchymal tumors in the gall bladder may be a more common finding at autopsy than reported in the literature, and in evaluating mesenchymal tumors in the gall bladder wall of dogs, both GIST and leiomyoma should be considered.

#### **D-046: INTRAHEPATIC BILE DUCT AND GALL BLADDER APLASIA IN MULTIPLE CANINE LITTERS WITH THE SAME PARENTAGE**

Karen Carlton, Margaret Stalker, Brandon Plattner

**Background:** Intrahepatic bile duct aplasia has been reported rarely in human children and is considered a congenital malformation incompatible with life. The condition has not been described in the veterinary literature. This case series involves three puppies from two sequential litters with the same parents, who were submitted for post mortem evaluation after dying at home at less than 5 weeks of age. All animals had a history of poor weight gain, as well as pale waxy and odorless feces. We describe complete intrahepatic bile duct aplasia, which was accompanied in all puppies by gall bladder absence/aplasia.

**Results:** On gross examination, all three puppies were in poor body condition. A shrunken tubular structure was identified in place of the gall bladder, and on cut section did not contain any bile. Histologically, all dogs had abnormal sinusoidal architecture with no appreciable bile ducts or ductules, and frequent small duplicated arterioles surrounding portal veins. In two puppies CK7 positive cells were not identified in any section of liver. In the other puppy there were rare individual or paired CK7-positive cells within portal tracts adjacent to limiting plate hepatocytes; however, no recognizable tubular structures were identified. Widespread intrahepatic canalicular cholestasis was identified in all three puppies.

**Conclusions:** We report a series of cases of intrahepatic bile duct and gall bladder aplasia in multiple litters of mixed-breed puppies with the same parentage. No predisposing cause was identified, but because all puppies were related, a genetic etiology is most likely.

#### **D-047: DECREASED EXPRESSION OF LEPTIN RECEPTOR IN CANINE HEPATOCELLULAR CARCINOMA: A PILOT STUDY**

Soo Hyeon Kim, Byung Joon Seung, Seung Hee Cho, Ha Young Lim, Jung Hyang Sur

**Background:** Leptin receptor (Ob-R), which interacts with leptin, is a member of class 1 cytokine receptor family. Leptin, produced by adipocyte, has been revealed to regulate energy homeostasis, and also the metabolism of glucose and lipid. Expression of leptin receptor was identified in various tumors, including human hepatocellular carcinoma, breast cancer, prostate cancer and canine mammary tumor. Also, leptin receptor has been found to be a contributing factor of tumor growth and metastasis.

**Objective:** The objective of the study was to identify the association between hepatocellular carcinoma and expression of leptin receptor.

**Methods:** Expression of leptin receptor was detected by immunohistochemistry on 8 normal liver tissues and 14 hepatocellular carcinomas. The samples including more than 5% of immunostained cells were considered as positive. Pearson's Chi-squared test was used for statistical analysis.

**Results:** Leptin receptor displayed cytoplasmic staining pattern in hepatic cells. Normal liver tissues positive for leptin receptor were 75% (6/8) and only 14.3% (2/12) of hepatocellular carcinomas were positive. The expression of leptin receptors was decreased in hepatocellular carcinoma than in normal liver tissues ( $P=0.008$ ). However, 2 hepatocellular carcinoma samples with leptin receptor positivity, showed aberrant overexpression in more than 50% of total cell population.

**Conclusions:** Leptin receptor could be a regulator of homeostasis in canine liver and may suppress tumorigenesis. Decreased expression level of leptin receptor may play as a carcinogenic factor, however, a larger scale study is indicated.

#### **D-048: SEVERE IDIOPATHIC LIVER AMYLOIDOSIS WITH SPONTANEOUS RUPTURE IN A HORSE**

Amanda Anderson, Kelsey Legendre, Frank Andrews, Fabio Del Piero

A 5-year-old Quarter Horse mare, used in cutting events, presented with a 2-week history of inappetence and lethargy just after entry into a competition. Clinical pathology revealed elevated liver enzymes, total bilirubin, and bile acids. Cytology was performed and showed amyloidosis with normal hepatocytes and occasional small lymphocytes. Liver biopsy further confirmed the diagnosis of amyloidosis. The horse was humanely euthanized due to poor prognosis and submitted to the Louisiana Animal Disease Diagnostic Laboratory for necropsy. The peritoneal cavity contained 5L of blood and there was diffuse icterus. The liver was pale, greatly enlarged, and friable, with an extensive left lobe visceral capsular tear. On histopathology, the hepatic parenchyma was effaced by myriads of ruptures appearing as variously sized blood filled cavitations bordered by fibrin. The adjacent Disse's space diffusely contained basophilic to acidophilic proteinaceous material (with Congo red stain affinity, and polarized light green birefringence) compressing atrophied hepatic cords. This amyloidosis was restricted to the liver. Localized and idiopathic liver amyloidosis with liver capsular rupture has been reported as a primary cause of hemoperitoneum in horses and has also been reported with splenic amyloidosis and subsequent splenic capsular rupture. Trauma during the cutting event is the most likely final insult that led to the rupture of the enlarged, friable liver with subsequent hemoabdomen.

#### **D-049: THE PROGNOSTIC VALUE OF HISTOLOGICAL CLASSIFICATION AND GRADING OF CANINE MAMMARY TUMORS: A COHORT STUDY IN SOUTH KOREA**

Byung-Joon Seung, Seung-hee Cho, Soo-Hyeon Kim, Jung-Hyang Sur

**Background:** Histopathology is cornerstone for diagnosing canine mammary tumors (CMTs). Recently, two classification systems (WHO classification of 1999 and Goldschmidt of 2011) have been used by pathologists. Although there are some survival studies of CMTs in the West, but large-cohort survival studies are lacking in the East. Furthermore, methods of raising dogs are different between the West and the East.

**Objective:** Our objective was to identify value of histological classification and grading of CMTs in the cohort of CMTs in South Korea.

**Method:** 2-year survival study that applied two classifications and one grading method was conducted to the cohort of 90 female dogs with CMTs in South Korea.

**Results:** Dogs with benign tumors (n=27), carcinoma arising in benign mixed tumors (n=16), complex carcinoma (n=15) and simple tubular carcinoma (n=7) had experienced prolonged survival. Those with simple tubulopapillary carcinoma (n=11), intraductal papillary carcinoma (n=2), and malignant myoepithelioma (n=1) had higher risk of tumor-related death. The prognosis was worse for anaplastic carcinoma (Survival time (ST) = 2 months), adenosquamous carcinoma (ST= 5 months), carcinosarcoma (ST = 8 months), comedocarcinoma (ST = 9 months) and solid carcinoma (Mean ST = 12 months). Those with malignant mesenchymal neoplasms (osteosarcoma (n=2) and fibrosarcoma (n=2)) had experienced prolonged survival.

**Conclusion:** Histological subtype was also significantly associated with survival in the cohort of the East. This study validated histological classification and histological grading in South Korea and provided information to be used in the clinical setting or as the basis for future prognostic studies.

#### **D-050: CANINE CONGENITAL MYOPATHY WITH MUSCLE SPINDLE EXCESS**

Matthew Lanza, Margret Casal, Amy Durham

**Background:** A 6-month-old male castrated American foxhound presented to the Veterinary Hospital of the University of Pennsylvania with a life-long history of hypermetric gait progressing to tetraparesis and severe generalized muscle wasting. Screening tests for amino acid, organic acid, and carbohydrate metabolic abnormalities were unremarkable. Urine glycosaminoglycan levels were within normal limits. Genetic testing for known canine Duchenne muscular dystrophy mutations was negative.

**Objective:** Our objective is to characterize the gross and histopathological features of this novel myopathy.

**Methods:** Routine necropsy and histopathology with hematoxylin and eosin staining was performed. Samples of skeletal muscle and nerve were submitted to the University of California San Diego Comparative Neuromuscular Laboratory for advanced staining including modified trichrome, periodic acid-Schiff, myofibrillar ATPases, esterase, NADH-tetrazolium reductase, acid phosphatase, alkaline phosphatase, oil red O, and protein A-peroxidase.

**Results:** Grossly, all skeletal muscles had diffuse pallor. Histologically, there was diffuse skeletal myofiber hypotrophy with predominance of Type I fibers and decreased numbers of large caliber nerve fibers. Histopathological examination of the biceps brachii muscles revealed increased numbers of muscle spindles. The spinal cord was unremarkable. Multiple heart valves had myxomatous degeneration.

**Conclusions:** The histopathological features, antemortem clinicopathological findings, and genetic testing results are not consistent with any specific disease described in dogs. A congenital myopathy with muscle spindle excess has been described in rare case reports in humans associated with a mutation in the HRAS gene as a possible variant of Costello (faciocutaneoskeletal) syndrome. This may represent the first such case described in a non-human species.

#### **D-051: GALECTIN-3 EXPRESSION IN CANINE ORAL MELANOMAS**

Thiago Henrique Vargas, Lidia Pulz, Daniel Ferro, Renata Sobral, Adriana Nishiya, Emerson Mota, Ricardo Strefezzi

**Background:** Galectin-3 (Gal-3) is a protein related to cell adhesion, apoptosis, angiogenesis, proliferation and differentiation. Its localization and/or expression level may be used as prognostic markers for human neoplasms such as thyroid, gastric and prostate tumors. Melanomas are the most common oral tumors in dogs. They can be classified as lowly or highly malignant, but there is a lack of consensus on prognostic indicators.

**Objective:** The aim of this study was to characterize Gal-3 expression in canine oral melanomas and to compare it with patient's post-surgical survival time, proliferation indexes, nuclear atypia and expression of apoptosis-related proteins.

**Methods:** Twenty-seven cases of canine oral melanomas were submitted to immunohistochemistry for the detection of Gal-3. Immunolabeling was evaluated in hot spot areas using quantitative and semiquantitative methods. Results were compared with Ki67 and mitotic indexes, nuclear atypia, and BCL2 and caspase-3 expressions. A minimum follow-up of 180 days was defined for censored cases.

**Results:** All tumors were positive for Gal-3 and overall immunolabeling was positively correlated with post-surgical survival and BCL2 expression. Nuclear immunostaining was negatively correlated with survival. No statistically significant correlations were found between Gal-3 expression and proliferation indexes, nuclear atypia or caspase-3 expression. Nuclear atypia was the best prognostic marker in this study regarding post-surgical survival.

**Conclusions:** We suggest that Gal-3 expression level and localization of the protein are potential prognostic markers for canine oral melanomas. The prognostic value of this protein should be further investigated in a larger number of cases.

#### **D-052: CANINE EXTRAHEPATIC BILIARY CARCINOID**

Jason Crawford, Michelle Rivard, Katie Barry, Bruce Williams

**Background:** An eight-year-old, intact male German Shepherd dog with a history of intermittent diarrhea and chronic weight loss that was unresponsive to medical management underwent an exploratory laparotomy. Identified on the apex of the gallbladder was a 3.0 x 2.0 x 1.0 cm, circumscribed umbilicated mass. The biliary mass and transmural gastrointestinal biopsies (stomach, duodenum, jejunum, and ileum) with sections of liver and pancreas were submitted for histopathology.

**Methods:** The submitted tissues were fixed in 10% neutral buffered formalin and processed routinely for H&E staining. Immunohistochemistry for synaptophysin and S-100 was performed on the biliary mass.

**Results:** The mass was well-circumscribed and composed of polygonal cells arranged in nests and packets that elevated the mucosa and expanded the submucosa of the gallbladder wall. Neoplastic cells had amphophilic granular cytoplasm with a low mitotic count and strong diffuse immunoreactivity to both synaptophysin and S-100, consistent with a neuroendocrine tumor (extrahepatic biliary carcinoid). There was a concurrent mild lymphoplasmacytic ileitis, and the remaining sections were unremarkable.

**Conclusions:** Canine carcinoids are uncommon neoplasms that originate from endocrine or paraendocrine cells, most often from the gastrointestinal tract and liver. There are only a few reports of this type of tumor involving extrahepatic biliary ducts. Carcinoids are generally slow growing malignancies that metastasize via lymphatics and hematogenous routes. Carcinoids should be included in the differential diagnosis for dogs with chronic gastrointestinal disease that is unresponsive to medical management.

#### **D-053: CUTANEOUS LIP AND NODAL T-CELL LYMPHOMA WITH GIANT CELLS IN TWO DOGS**

Macallister Harris, Caitlyn Martinez, Emily Rout, Kelly Hughes, Kari Frankhouse, Anne Avery, Connie Stevenson, Sushan Han, Amy MacNeil

**Background:** Epitheliotropic lymphoma in dogs is predominantly a CD8+ T-cell phenotype with variable lymphocyte size.

**Objectives:** To describe the clinical outcome, cellular morphology and phenotypic abnormalities of lymphoma affecting the lip and lymph node in two dogs.

**Methods:** Immunohistochemistry (IHC) and/or immunocytochemistry (ICC) was performed for CD3, PAX5, factor-VIII related antigen, MUM1, CD18 and CD204. Flow cytometry was performed on both cases.

**Results:** Both dogs presented with a lip mass and enlarged ipsilateral mandibular lymph node. Cytology and/or histology of both sites revealed a monomorphic small to intermediate-sized lymphoma with frequent giant karyomegalic cells (100 to 200 um in diameter) with variably shaped nuclei and coarsely granular chromatin. The expression of CD3 identified a T-cell phenotype in both dogs. The atypical giant cell population was immunoreactive for CD3, and did not express PAX5, factor-VIII related antigen, MUM1, CD18 or CD204. Flow cytometry of both cases demonstrated an expansion of CD8+,

CD3+, CD45+ T cells with low MHC class II expression. Histology on one case revealed epitheliotropism with nodal involvement that could not clearly be classified into a WHO subtype. The dogs were treated with either excisional surgery or chemotherapy, and have maintained complete remission for 5 and 6 months, respectively.

**Conclusions:** Immunohistochemistry and immunocytochemistry identified a T-cell lymphoma with atypical giant cells affecting the lip and lymph node. Flow cytometry demonstrated a CD8+ T-cell immunophenotype. The presence of these giant cells and similar features in two cases may represent an unrecognized variant of T-cell lymphoma.

**D-054: ACUTE LYMPHOCYTIC LEUKEMIA (ALL) IN A GOLDEN RETRIEVER DOG**  
Stephanie Muller, Graham Stock, Jerome Braun, Margotte Landry

**Background:** Acute lymphocytic leukemia (ALL) is an uncommon lymphoid malignancy in dogs, and its diagnosis can be challenging. A 3-year-old female Golden Retriever dog was presented for weight loss, lack of energy and appetite. The patient had pale mucous membranes and weak pulse. There was a transient clinical improvement with doxycycline, imidocarb and corticosteroid therapy, with further deterioration after 2 weeks. The dog died with moderate non-regenerative anemia, marked thrombocytopenia and neutropenia and moderate lymphocytosis (25 x 10<sup>9</sup>/l leukocytes with 99% lymphocytes). ALL was tentatively diagnosed based on finding immature appearing lymphocytes on blood smear.

**Objective:** Our objective was to confirm the clinical hypothesis of acute lymphocytic leukemia.

**Methods:** Peripheral blood smears, stained with May-Grünwald Giemsa (MGG), were reviewed by board certified veterinary clinical and anatomical pathologists. Post-mortem examination only showed enlarged spleen. Bone marrow smears stained with MGG and histopathologic splenic sections stained with H&E were examined. PCR for antigen receptor rearrangement (PARR) on formalin-fixed splenic tissue was performed.

**Results:** Peripheral blood smear findings included moderate leukocytosis with 23% small and 76% intermediate immature lymphocytes. Bone marrow aspirate and splenic sections showed small to medium sized lymphoid cells. The findings were consistent with acute lymphocytic leukemia. PARR gave a clonal T cell receptor result.

**Conclusions:** The dog, diagnosed with ALL, survived 4 weeks after initial clinical signs. Examination of peripheral blood smears is a reliable diagnostic method to diagnose ALL in dog. Our results indicate that PARR can be a useful diagnostic tool to detect canine lymphoid malignancy post-mortem.

**D-055: HISTOPATHOLOGICAL GRADING AND IMMUNOHISTOCHEMICAL DETECTION OF GALECTIN-3 IN CANINE CUTANEOUS SQUAMOUS CELL CARCINOMAS**

Gabriela Marques, Thiago Henrique Vargas, Lidia Pulz, Greice Huete, Karine Cadrobbi, Silvio Freitas, Ricardo Strefezzi

**Background:** Squamous cell carcinomas (SCC) are common skin neoplasms in dogs and cats, usually associated with exposure to UV radiation. Broder's grading system is used to subclassify this neoplasm in 4 grades and is considered an important prognostic indicator. There is increasing evidence that Galectins play important role in cancer. Galectin-3 is one of the most important members and is known to interfere with apoptosis, tumor growth, invasion and metastasis, thus influencing aggressiveness.

**Objective:** The objective of the present study was to characterize the expression of Gal-3 in canine SCCs and to investigate its expression in cutaneous SCCs of different histopathologic grades.

**Methods:** Twenty-two canine cutaneous SCCs from 15 dogs were reviewed and graded using Broder's grading system, by three simultaneous observers. Gal-3 expression was detected immunohistochemically using a primary mouse monoclonal anti-Gal-3 antibody (ab2785, Abcam). The immunostaining was quantified in five high-power fields from hot spot areas using a semiquantitative method based on scores for the percentage of positive cells and staining intensity.

**Results:** All but one of the tumors were positive for Gal-3. The immunostaining was cytoplasmic and/or nuclear, and tumors from the same animal showed different scores for Gal-3. The positivity for Gal-3 did not reveal statistically significant differences between histopathologic grades.

**Conclusions:** Our preliminary results show that Gal-3 is expressed by canine cutaneous SCC cells and immunostaining is highly variable. Ongoing studies in our laboratory aim to investigate Gal-3 expression in a larger number of cases, as well as differences between nuclear and cytoplasmic expressions.

#### **D-056: OVARIAN TUMOUR IN CAT ARISING FROM AN OVARIAN REMNANT: DESCRIPTION OF 2 CASES**

Elena Riccardi, Sophie Le Calvez, Jenny McKay

**Background:** Tumours of the feline ovary are rare. This is most likely a reflection of the high percentage of neutered cats examined by veterinarians. Ovarian tumours are classified into epithelial, germ cell or sex cord-stromal tumours, based on their cell of origin. Ovarian remnant syndrome (ORS) is a condition that occurs when ovarian tissue is not completely removed during ovariectomy or ovariohysterectomy. This syndrome is reported in both dogs and cats and is associated with clinical signs of oestrus. Neoplasia might also develop within the residual tissue.

**Methods:** Two neutered female cats were presented with clinical signs of oestrus. Exploratory laparotomy revealed the presence of a mass in the region of the left ovarian pedicle in both cases. Each mass was removed and submitted for histological examination. Immunohistochemical analysis was also performed.

**Results:** Histologically, a neoplastic proliferation of polygonal cells arranged in sheets or lining follicular-like structures was noted in both samples. A morphological diagnosis

of granulosa cell tumour was made for both masses. In one case, the neoplastic cells showed a strong positive immunoreactivity for inhibin.

**Conclusions:** The clinical signs resolved following surgical excision of the remaining ovarian tissue. To our knowledge, this is the first report of ovarian tumours in cats arising from ovarian remnants. These should be considered as differentials when neutered cats present with signs of oestrus.

#### **D-057: IMMUNOHISTOCHEMISTRY ANALYSIS OF CHROMATOPHOROMAS IN TWO SPECIES OF TELEOSTS (CYPRINUS CARPIO AND CARASSIUS AURATUS)**

Wesley Siniard, Matthew Sheley, Esteban Soto

**Background:** Eight fish from different facilities presented to the University of California Davis Veterinary Medical Teaching Hospital over a six year period with similar masses which were all diagnosed as pigment cell tumors/chromatophoromas. Three of the submissions were necropsies, and the other five were biopsy samples. All of the neoplastic lesions were located along the dorsum or dorsal skull/periorbital region of the fish, and the lesions were variably pigmented.

**Objective:** Our objective was to compare the histologic morphology of the neoplasms, and to identify a useful immunohistochemistry assay to diagnose chromatophoromas in teleosts, specifically when the chromatophoromas are poorly pigmented.

**Methods:** Hematoxylin and eosin (H&E) as well as three immunohistochemistry assays (PNL-2, Melan-A, and SOX-10) were performed on formalin-fixed, paraffin-embedded samples of each mass. The histologic appearance of each neoplasm on H&E was described, and the level of immunoreactivity for each immunohistochemistry assay was graded on a scale of 0 to 3, with 0 being non-immunoreactive, 1 being mildly immunoreactive, 2 being moderately immunoreactive, and 3 being strongly immunoreactive.

**Results:** The neoplasms examined shared similar histologic features. PNL-2 exhibited mild to moderate immunoreactivity in seven cases and resident chromatophores were also immunoreactive. Melan-A exhibited mild to moderate immunoreactivity in four cases, and SOX-10 did not exhibit immunoreactivity in any of the cases examined.

**Conclusions:** PNL-2 may be a useful marker in teleosts to distinguish tumors of chromatophore origin. Melan-A may also be useful, but SOX-10 is likely not a useful marker for these neoplasms in teleosts.

#### **D-058: ENDOMETRIAL DISEASES IN SIX CATS WITH CLINICAL AND HISTOPATHOLOGICAL FEATURES RESEMBLING ATYPICAL ENDOMETRIAL HYPERPLASIA OF HUMANS**

Satoshi Suzuki, Hidetomo Kitamura, Kotaro Hayashi, Tomomi Nakashima, Masaru Okamura, Katsuaki Shirai, Takuro Kariya

Endometrial adenocarcinoma in cats is highly metastatic and invasive with recurrence or metastasis tending to occur early after resection. In humans, endometrial

adenocarcinoma must be distinguished from atypical endometrial hyperplasia. The present report describes the histopathological features of an endometrial lesion in six cats, which histopathologically resembled human atypical endometrial hyperplasia and had a good prognosis via ovariohysterectomy. Six cats were presented for hemorrhagic or purulent vaginal discharge, emotional behavior, or medical health check. The ages ranged from 11 months old to 13 years old (median: 10.4 years). Four cases affected Bengal cats (three of these being relatives) and two cases were observed in mixed breeds. Grossly, one cat presented with papillomatous nodules while no masses were observed in the other five cases. Three cases presented with pyometra. Histopathologically, proliferation of endometrial epithelial cells without atypia was observed in all cases. In some areas of some cases cells had increased atypia and were arranged in stratified layers, forming irregular ducts and papillary structures. No apparent invasive behavior or vascular invasion was found. Based on these findings, cases were diagnosed as non-invasive or early-stage adenocarcinoma. Atypical endometrial cells extended to the surgical margins in two cases. In one of them, the lesion was completely excised after extended resection. All cats survived and the median survival time was 378 days (range: 14 to 744 days). The pattern of distribution of histopathological endometrial changes and the non-invasive behavior in these feline cases is similar to cases of atypical endometrial hyperplasia of humans.

#### **D-059: MALIGNANT MELANOMA ARISING FROM THE EYE IN A DUCK (ANAS PLATYRHYNCHOS)**

Alonso Reyes-Matute, José-Antonio Ruiz-Remolina, Sandra Díaz-Yucupicio

Ocular and eyelid neoplasia is seldom reported in aviary species, but several tumors are known to arise from the eye. In birds, melanocytic tumors are neoplasms of uncommon occurrence and typically located on the face and beak. A 4-year-old, female, duck (*Anas platyrhynchos*), was presented due to a mass in the right eye. A cytology sample was obtained and revealed findings suggestive of a melanocytic tumor. Enucleation was performed 7 days later but histopathology was not performed. Three weeks later, the duck presented with exophthalmos of the left eye and blindness. The duck developed progressive behavioral changes, depression, anorexia and weight loss. Euthanasia was elected and a full necropsy was performed. Transverse sections of the whole head showed a locally invasive firm, pigmented, multilobular mass. This mass was encompassing the whole right side of the head and most of the left side as well (including the orbit and soft tissues). It partially occupied and replaced the nasal cavity and multifocally infiltrated the brain and soft tissues of the neck. On histopathology, the lesion was consistent with a heavily pigmented malignant melanoma. Although rare, malignant melanomas in the order Anseriformes have been described affecting the eyeball, originating from the thoracic inlet and arising from the dorsal surface of the bill. In all previously reported cases and the one described here, tumor behavior was aggressive. Although multi-organ metastasis has been reported, no distant metastases were identified in this particular case.

#### **D-060: CHOLANGIOCELLULAR CYSTADENOCARCINOMA IN A BLACK TAILED PRAIRIE DOG: A CASE REPORT**

Seung Hee Cho, Byong Joon Seung, Soo Hyeon Kim, Jung Hyang Sur

A 6-year-old intact female black tailed prairie dog presented lethargy and abdominal distention. A 2.5×2×2 cm cystic mass was detected in the liver during a surgical procedure. The animal died shortly after surgery. On histological examination, the mass was composed variably sized cysts lined by a single to multiple layers of neoplastic biliary epithelium. Neoplastic cells were cuboidal to polygonal with round nuclei. Moderate anisokaryosis was observed and there were few mitotic figures. The tumor cells were positive for cytokeratin 7 and claudin-7 and negative for HepPar1. Based on histopathologic and immunohistochemical features, the tumor was diagnosed as a cholangiocellular cystadenocarcinoma. To the authors' knowledge, this is the first case of cholangiocellular cystadenocarcinoma in black-tailed prairie dogs.

#### **D-061: ORAL MELANOMA WITH PSEUDOVASCULAR SPACES IN THREE DOGS**

Gisela Martinez-Romero, Richard Weiss, Kellye Joiner, Joseph Newton

Melanomas with pseudovascular spaces have been described in human melanocytic nevi and cutaneous melanomas. The cause of this change is unknown but it has been attributed to an artifact of local injection or tissue processing. The spaces resemble vascular or lymphatic vessels and may contain blood or nests of melanocytes. The current report describes the histologic features of oral melanomas with pseudovascular spaces in three dogs (Case 1: A 9-year-old castrated male Shih Tzu; Case 2: A 13-year-old spayed female American Cocker Spaniel; Case 3: A 9-year-old intact female Chow mix). Case 1 presented with a recurring oral mass over the right maxilla with amelanotic melanoma and hemangiosarcoma as differential diagnoses; case 2 presented to the Auburn University Veterinary Teaching Hospital Oncology services for examination of a left mandibular mass in the oral cavity; and case 3 had a history of a large oral mass. The neoplasms had histologic features similar to those of oral melanomas, but accompanied by vascular-like spaces lined by neoplastic cells, that were multifocally filled with an eosinophilic material, few erythrocytes and individual neoplastic melanocytes. Neoplastic cells lining the pseudovascular spaces displayed moderate to strong cytoplasmic immunoreactivity for Melan-A and S100, and were negative for CD31. To our knowledge, no cases of oral melanoma with pseudovascular spaces have been reported in dogs. It is important to be aware of this histologic feature in oral melanomas to avoid potential misdiagnosis.

#### **D-062: OH DEAR HEART! A CASE OF A PRIMARY INTRA-ATRIAL PARAGANGLIOMA IN A DOG**

Monica Ronderos, Sophie Aschenbroich, Gillian Shaw

A 16-year-old castrated male, Brittany spaniel dog was euthanized following an approximately 4-year history of a slow-growing right atrial mass and intermittent episodes of ventricular arrhythmia, paroxysmal atrial tachycardia, and mild pulmonary hypertension. Echocardiographic examination initially identified an intra-atrial mass, which measured 1.5 cm in diameter. At the time of the final cardiology appointment, shortly before euthanasia, the mass occluded 80% of the right atrium. At necropsy, a 7.0 x 8.0 x 3.5 cm, multilobulated, tan to red, semi-firm mass was identified to protrude from the endocardial surface of the right atrium. Additional masses included a 0.9 x 0.7 x 0.5 cm dark red mass on the endocardial surface at the base of the right atrial

appendage and a 1.0 x 0.9 x 0.6 cm tan mass in the papillary muscle of the left ventricle. Histologically, these masses consisted of polygonal neoplastic cells with granular cytoplasm, separated into nests and packets by a fine fibrovascular stroma. Immunohistochemical staining confirmed that the neoplastic cells expressed chromogranin A and synaptophysin. Based on immunohistochemical features and lack of involvement of the aortic body, the right intra-atrial mass was diagnosed as a paraganglioma with extension to the right atrial appendage and left ventricular wall. Primary intracardiac paragangliomas represent rare neoplasms, with only three cases having been reported in the veterinary literature. This case report provides a detailed review of the distribution of normal paraganglia in tissues as well as clarifications of the terminology of neuroendocrine neoplasms arising within or near the heart base.

### **D-063: EXPRESSION OF 2',3'-CYCLIC NUCLEOTIDE 3'- PHOSPHODIESTERASE (CNPase) IN CANINE OLIGODENDROGLIOMAS**

Flaviu Tabaran, Elizabeth Pluhar, Michael O'Sullivan

**Background:** Immunohistochemical identification of neoplastic oligodendrocytes remains a major challenge and a diagnosis-limiting factor in poorly differentiated or mixed subtypes of glial tumors in which co-expression of several glial and neural markers is frequently present. A promising oligodendroglial marker in both tumoral and normal oligodendrocytes is 2',3'-cyclic nucleotide 3'- phosphodiesterase (CNPase), which is expressed early in myelination and responsible for hydrolyzing cyclic nucleotides to monophosphates.

**Objective:** To evaluate the expression of CNPase in canine oligodendrogliomas, and to assess the robustness of this marker in samples following long-term fixation.

**Methods:** A retrospective analysis was carried out in oligodendrogliomas obtained by surgical biopsy from 14 dogs; whole brains obtained at necropsy were available for 11 of these cases.

**Results:** CNPase was diffusely expressed in all biopsy samples of oligodendrogliomas, albeit in variable amounts in different regions of the tumor. The immunoexpression was mild to intense, granular-fibrillar, diffuse-cytoplasmic or perimembranous. Extracellular myxoid material separating the tumoral oligodendrocytes was CNPase-negative. Within brains collected at necropsy, CNPase expression was absent in 6 cases and minimal to mild, multifocal, for the other 5 cases, with cytological distribution as already described.

**Conclusions:** Our observations indicate that CNPase is a sensitive marker for tumoral oligodendrocytes, and a useful antigen for evaluating biopsy material from oligodendrogliomas. The very high expression of CNPase in normal neural tissue limits its utility in assessing tumor margins. The loss of immunolabeling following long-time fixation limits the utility of CNPase in assessing necropsy samples.

#### **D-064: INFLUENCE OF GENDER AND AGE FLUCTUATIONS ON CANCER INCIDENCE IN A GROUP OF 7,780 SOUTHERN BRAZILIAN DOGS SUBMITTED TO NECROPSY OVER FIFTY YEARS (1964-2013)**

Mariana Flores, Alfredo Cezar, Douglas Lorensetti, Glaucia Kommers, Rafael Figuera

**Background:** Cancer is among the most common causes of canine mortality, yet its incidence in dog populations remains largely unexplored.

**Objective:** To analyze the incidence and epidemiology of cancer in a group of dogs submitted to necropsy over 50 years.

**Methods:** Files from dogs submitted to necropsy (1964-2013) at a Veterinary Laboratory in southern Brazil were searched for data regarding gender, age, breed and cause of death. Information was analyzed per decade. Epidemiological features were analyzed using Chi-square test and Spearman correlation with 99% confidence level.

**Results:** Over 50 years, 7,780 dogs were submitted to necropsy, of which 867 (11.1%) died of cancer. Data analysis revealed an increase in the proportion of females ( $p=0.003$ ) (from 33.3% [1964-1973] to 53.1% [2004-2013]) and in the median age of death (from 2 [1964-1973] to 5 years [2004-2013]) over the decades. Cancer incidence (CI) increased mainly over the last two decades: [4,2% (1964-1973); 3,5% (1974-1983); 5,2% (1984-1993)]; 12.8% (1994-2003) and 16.5% (2004-2013) ( $p<0.01$ ). The disease was more frequent ( $p<0.0001$ ) in females (13.7%) than in males (8.8%). Mammary carcinoma (24,5%), lymphoma (8,8%), osteosarcoma (7,8%) and cholangiocarcinoma (5,5%) were the most frequent tumors in dogs. A positive correlation was found between age and cancer ( $p<0,0001$ ), with moderate ( $r=0.5$ ) and low ( $r=0.3$ ) levels regarding females and males, respectively.

**Conclusion:** CI has increased in this group of dogs over 50 years. This growth was affected by gender and age fluctuations along the years. Another relevant finding was the high prevalence of cholangiocarcinoma, an uncommon type of canine cancer.

#### **D-065: SQUAMOUS CELL CARCINOMA WITH CLEAR CELL DIFFERENTIATION IN AN EQUINE EYELID**

Leah Stein, Dodd Sledge, Rebecca Smedley, Matti Kiupel, Tuddow Thaiwong

A 15-year-old Miniature horse mare had a six-month history of an ulcerated mass on the right lower eyelid. An incisional biopsy and a subsequent excisional biopsy were submitted to the Michigan State University Veterinary Diagnostic Laboratory for microscopic evaluation. Histologically, the incisional biopsy was composed of sheets of large neoplastic vacuolated polygonal cells. Few regions contained poorly differentiated neoplastic round to basaloid cells that rimmed the sheets of highly vacuolated polygonal cells. Both vacuolated and basaloid cells exhibited strong perimembranous immunoreactivity for E-cadherin. Vacuolated polygonal cells were histochemically negative for periodic acid-Schiff, mucicarmine, and oil red O, consistent with a diagnosis of poorly differentiated carcinoma. Within the excisional biopsy specimen, there were anastomosing cords and nests of neoplastic squamous epithelial cells along the

periphery of the mass. The neoplastic squamous epithelial cells surrounded central vacuolated cells. These findings are consistent with a squamous cell carcinoma with clear cell differentiation. In addition, in the adjacent dermis, there was solar elastosis suggestive of UV damage. A clear cell variant of squamous cell carcinoma is a rare entity in humans that has not been previously described in animals and is often associated with chronic UV exposure.

#### **D-066: IMMUNOHISTOCHEMICAL PROFILES OF LINGUAL GRANULAR CELL TUMORS IN 10 DOGS**

Gordon Ehrensing, Matti Kiupel, Dodd Sledge

**Background:** Granular cell tumors are a well-recognized entity of the canine tongue. Neoplastic cells are round to polygonal and contain numerous amphophilic intracytoplasmic granules, which are frequently periodic acid–Schiff (PAS) positive and diastase resistant. Histogenesis of these tumors remains incompletely understood. This diagnosis may incorporate multiple morphologically convergent entities.

**Objective:** Our objective was to evaluate the histogenesis of canine lingual granular cell tumors.

**Methods:** Ten lingual canine masses previously diagnosed as granular cell tumor were retrieved from the MSU VDL biopsy archive. Diagnoses were confirmed in H&E stained sections. Histochemistry for PAS and immunohistochemistry for S100, muscle-specific actin, desmin, CD204, CD18, allograft inflammatory factor 1, uncoupling protein 1 (UCP1), and CD31 were performed.

**Results:** All tumors arose within lingual stroma of adult dogs greater than 6 years of age. There was moderate morphologic variation between specimens. 1/10 tumors were PAS positive, and 1/10 tumors were immunoreactive for desmin. Eight tumors including both the PAS and desmin positive neoplasms were immunoreactive for UCP1. Neoplastic cells within all cases were not immunoreactive for remaining markers.

**Conclusions:** Lingual granular cell tumors in dogs frequently lack PAS positivity and S100 immunoreactivity. Immunoreactivity for desmin in one tumor is supportive of a neoplasm of muscle origin. While not exclusive to brown fat, immunoreactivity of neoplastic cells for UCP1 in the absence of additional immunoreactivity may suggest a subset of tumors are hibernomas. Findings support the proposition that granular cell tumors may have multiple distinct histogeneses; prognostic significance of this variance in histogenesis is unclear.

#### **D-067: METASTATIC SARCOMA AND ACQUIRED PORTOSYSTEMIC SHUNTS IN A WATER MOCCASIN (AGKISTRODON PISCIVORUS)**

Leslie Charles, Robert Ossiboff, Lisa Farina

An adult male water moccasin (*Agkistrodon piscivorus*) from a zoological institution was presented for necropsy after spontaneous death. The snake had a history of pericloacal granulomas and a palpable liver mass. Prior bloodwork included elevated fasting bile acids and hypoglycemia, suggestive of liver disease. The snake had been eating well,

but was noticeably cachectic. At necropsy, a sarcoma was identified completely encapsulating the liver. Multiple tortuous blood vessels, up to 2 mm in diameter, were present on the serosal surface of the stomach and proximal small intestine, consistent with acquired portosystemic shunts. Neoplastic mesenchymal cells frequently contained single or multiple discrete, round, clear vacuoles, and there was marked pleomorphism. Masses of neoplastic cells similar to those encapsulating the liver were also identified in the heart, aorta, kidney, adrenal gland, fat bodies and skeletal muscle of the body wall. The neoplasm was most consistent with a liposarcoma, which may have originated in the fat bodies and subsequently metastasized to surrounding and distant visceral organs. Encapsulation of the liver by the neoplasm and subsequent occlusion of the portal vein likely resulted in portal hypertension and the formation of acquired shunts. To our knowledge, this is the first report of acquired portosystemic shunts in a reptile, and this is an unusual mechanism for development of portal hypertension in any species.

#### **D-068: CONGENITAL MELANOMA IN THE VERTEBRAL CANAL IN A SAANEN GOAT**

Devinn Sinnott, Kevin Woolard, Verena Affolter

A 9-day-old, male, Saanen goat presented to the University of California, Davis Veterinary Medical Teaching Hospital for bilateral hind limb paresis following dystocia. Both hind limbs ultimately progressed to complete paralysis with loss of nociception, and humane euthanasia was elected. On postmortem examination, the spinal cord from T5 caudally was circumferentially surrounded by dark red to tan, soft tissue within the subdural space. Histologically, this tissue was composed of pleomorphic, polygonal to spindloid cells containing sparse, dark brown, intracytoplasmic pigment granules. A Fontana-Masson stain and immunohistochemistry with SOX10 and PNL2 confirmed the melanocytic origin of these cells. Melanomas are rare in small ruminants, and congenital melanoma has not been reported in sheep and goats. Congenital melanoma with metastasis to the vertebral canal has been reported in humans and calves, but primary congenital vertebral canal melanoma has not been documented. This case describes the first primary congenital vertebral canal melanoma in a goat.

#### **D-069: RETROSPECTIVE PATHOLOGICAL REPORTS OF SPLENIC LESIONS IN DOMESTIC HAMSTER**

Yun-Chieh Tuan, Ruo-Chan Wang, Ju-Po Kao, Kimimasa Takahashi, Jiunn-Wang Liao

A total of 15 splenic biopsy specimens in 212 cases of hamsters from the Division of Wild (Exotic) Animal Medicine, Veterinary Medical Teaching Hospital, National Chung Hsing University, between 2010 and 2017 were studied for the retrospective pathological study. The incidence in the spleen was 7.1% (15/212), including 15 *Phodopus* hamsters. Ten neoplasms and 5 non-neoplastic lesions in the *Phodopus* hamsters occurred. For neoplastic lesions, the prevalence in *Phodopus* hamsters was higher than in Syrian hamsters (found 3 cases in VMTH, NCHU), which are similar to laboratory hamsters. In *Phodopus* hamsters, the most common tumors are histiocytic sarcoma (HS), lymphoma, malignant fibrous histiocytoma (MFH) and hemangiosarcoma. In addition, hemangiosarcoma and histiocytic sarcoma were found

in Syrian hamsters. The non-neoplastic lesion was a fibrotic nodule. The mean age of affected hamsters was 16.6 months, and females were affected more than males. In immunohistochemical staining, histiocytic sarcoma and malignant fibrous histiocytoma were positive for lysozyme, lymphoma was negative for CD79a and CD20, one case of lymphoma was positive for CD3, and hemangiosarcoma was positive for Von Willebrand factor (VWF). In terms of non-neoplastic lesions, the most common are fibrotic nodules and all occur in aging female hamsters. The nodules consist of collagen fiber that can be identified with Masson's trichrome stain, and are related to repairing of trauma in the spleen. To our knowledge, there have been no retrospective pathological reports of splenic lesions in domestic hamsters. Therefore this study can provide some valuable analytical data about spleens in hamsters.

#### **D-070: GANGLIONEUROMA OF THE GALLBLADDER AND BILIARY TREE IN A TWO-YEAR-OLD DOG RESULTING IN EXTRAHEPATIC BILIARY OBSTRUCTION**

Sunil More, William Craft

**Clinical Presentation:** A two-year-old male Labrador retriever with a history of inflammatory bowel disease presented for acute vomiting, icterus, and cranial abdominal pain. An abdominal ultrasound showed evidence of biliary obstruction. An exploratory laparotomy revealed a severely distended, white, thickened gallbladder and biliary tree, an enlarged major duodenal papilla, and a large amount of mucoid material within the gallbladder. The severe thickening of the biliary tree persisted after flushing, and euthanasia was elected. The biliary tree was dissected post-mortem, and submitted for surgical pathology.

**Microscopic Description:** The lamina propria of the biliary tree and gallbladder wall is infiltrated by a poorly demarcated, low to moderately cellular mass composed of neoplastic nerve cell bodies (ganglia) that are within an abundant fibrous stroma that contains many nerve fibers and large nerve bundles and fascicles. The ganglion cells are large, oval to polygonal, have abundant eosinophilic cytoplasm with Nissl substance, have distinct cellular borders, and are scattered and form small aggregates within the stroma. The large vesicular nuclei are irregularly round to oval and have a single large, prominent nucleolus. There is mild anisocytosis and anisokaryosis and 3 mitoses are observed in ten 400x fields (2.37mm<sup>2</sup>).

**Morphologic Diagnosis:** Ganglioneuroma, biliary tree and gallbladder

**Conclusion:** Immunohistochemistry supported the diagnosis of ganglioneuroma throughout the biliary tree and gallbladder. Ganglioneuromas are rare in this location, with few reported cases. To the authors' knowledge, the extensive involvement of the biliary tree and gallbladder, as seen in this case, has not been previously reported.

#### **D-071: IMMUNOHISTOCHEMISTRY AND ULTRASTRUCTURE OF EPITHELIAL MESOTHELIOMA IN A RED SINDHI HERD FROM BRAZIL**

Daniel Guimarães Ubiali, Ileana Miranda, Yasmin Daoualibi, Samara Lopes, Marilene Farias Brito, Anibal Armien

**Background:** Mesothelioma is a neoplastic proliferation originating from the parietal and visceral serous surfaces of the thoracic, abdominal, or pericardial sac; it is rarely reported in adult cattle. Exposure to asbestos dust has been associated with mesotheliomas etiology in human.

**Objective:** To describe an epithelial mesothelioma in four adult Red Sindhi cows euthanized after three to eight months of progressive emaciation, subcutaneous edema of the lower body regions and abdominal distension.

**Results:** On necropsy, there was a large amount of serous fluid in the abdominal cavity. Throughout the parietal and visceral serosa of multiples organs there was multifocal to coalescing, yellow, firm, sessile, nodular tissue proliferation. There were also free tissue nodules floating within fluid. Histologically, the masses were composed of a single cell-layer of cuboidal columnar cells supported by a dense fibrovascular stroma that formed small to large papillary proliferation. Neoplastic cells had a 2:1 cytoplasm:nucleus ratio. The nuclei were small with fine stippled chromatin and one to three nucleoli. Mitosis figures vary from 5 to 10 nt per 20 powered field. A small to moderate number of lymphocytes and plasma cells infiltrated the stroma. The neoplastic cells expressed cytoplasmic immunoreactive only for cytokeratin. The transmission electron microscope demonstrated neoplastic cuboidal cells with delicate microvilli and tight and anchoring junctions. Within the cytoplasm was a moderate amount of loose aggregate of intermediary filament with small mitochondria.

**Conclusions:** The cell morphology, immunohistochemistry, and ultrastructural findings were consistent with epithelial mesothelioma. The etiology remains undetermined. A familial predisposition is suspected.

#### **D-072: HOLOPROSENCEPHALY IN AN AFRICAN GREEN MONKEY (CHLOROCEBUS AETHIOPS SABAEUS)**

Caralyn Labriola, Rachel Andrews, Matthew Jorgensen, Nancy Kock

Holoprosencephaly is a structural midline developmental defect in the brain, which is frequently accompanied by facial deformities and other congenital malformations. Herein we detail a case which closely resembles semilobar holoprosencephaly in humans with many accompanying developmental abnormalities in a full term stillborn vervet/African green monkey (*Chlorocebus aethiops sabaeus*). The animal was microcephalic, had irregular fusion of the metopic suture of the skull with trigonocephaly, and had severe facial midline deformities including unilateral microphthalmia, arrhinia, cheiloschisis, and palatoschisis. The rostral aspect of the longitudinal fissure of the brain was absent, and at the level of the thalamus, although present, was irregularly shaped, and was incomplete ventrally. The lateral ventricles were dilated and the corpus callosum was absent. Histologically, structures along the midline including the fornix, caudate nucleus, rostral striatum, and putamen were absent. The cranial cingulate sulcus, anterior commissures, left thalamic nucleus, and temporal, parietal, and occipital lobes were markedly reduced in size. Other findings include enlargement of the heart, liver, kidneys, and ovaries, and megacolon. This case of holoprosencephaly appears to be a first report in African green monkeys.

### **D-073: FIBROCARILAGINOUS EMBOLIC ENCEPHALOMYLOPATHY OF THE CERVICAL SPINAL CORD INVOLVING THE CEREBELLUM IN A CAT**

Kazuki Okada, Teita Ishizaki, Yumiko Kagawa

A 9-year-old castrated male domestic short-haired cat exhibited acute-onset paralysis of four limbs. Magnetic resonance imaging revealed an intramedullary lesion at C3 and the vermis and right hemisphere of the cerebellum, which were hyperintense on T2-weighted imaging. The following day, the cat was euthanized due to poor prognosis and postmortem examination was conducted. Transverse sectioning of the spinal cord revealed gross malacia extending from the C2 to the C3. However, changes in the cerebellum were subtle. Histologically, severe and regionally extensive necrosis of the bilateral ventral gray matter and dorsal gray matter on the right side was noted at the C2 to C3 level. Adjacent white matter tracts exhibited axonal Wallerian degeneration. The ventral spinal artery and intramedullary arteries contained homogeneous, faintly basophilic material. The material was confirmed to be fibrocartilage by metachromatic staining with toluidine blue. Fibrocartilaginous emboli were also found in the blood vessels of the pia mater, predominantly in the vermis and right hemisphere of the cerebellum. The cerebellar cortex surrounding the pia mater showed diffuse edema, and Purkinje cells were decreased in number and had hypereosinophilic cytoplasm. Fibrocartilaginous embolism (FCE) has been well-documented in dogs and is sporadically reported in cats. In cats, these lesions are exclusively within the spinal cord. In this case, the emboli may have traveled from the ventral spinal artery to the caudal cerebellar artery via the basilar artery. To our knowledge, this is the first report of multifocal FCE involving both the cerebellum and cervical spinal cord in a cat.

### **D-074: GLOBOID CELL LEUKODYSTROPHY IN THREE RELATED BORDER COLLIE MIXED-BREED PUPPIES**

Caitlin Burrell, Miranda Vieson, Devon Hague, Barbara Kompare

**Background:** Three five-month-old, related, Border Collie mixed-breed puppies presented for necropsy due to progressive ataxia, incontinence, and head tremors. Antemortem PCR testing for Imerslund-Gräsbeck syndrome and serologic testing for *Toxoplasma gondii* and *Neospora caninum* was negative and cobalamin levels were high. MRI of the brain and spinal cord of one puppy indicated symmetrical cortical and cerebellar white matter hyperintensity and contrast enhancement, suggestive of leukoencephalopathy due to an inflammatory or metabolic disease process. Treatment with doxycycline, clindamycin, and prednisone was unsuccessful. During gross examination, no lesions were identified in the central or peripheral nervous systems. On histopathology, the white matter and perivascular spaces in the brain and spinal cord contained numerous plump macrophages with abundant amphophilic fibrillar to flocculent cytoplasm (globoid cells).

**Objective:** Our objective was to identify “globoid cells” within the brain and spinal cord white matter and confirm the diagnosis of globoid cell leukodystrophy in a previously unreported canine breed mix.

**Methods:** A gross examination was completed on all three puppies. Formalin-fixed, paraffin-embedded sections of the cerebrum, cerebellum, midbrain, spinal cord, and peripheral nerves were stained with hematoxylin and eosin.

**Results:** Globoid cells were present within the white matter and perivascular spaces of all three puppies. Cellular cytoplasm stained positively with Periodic Acid-Schiff stain. Globoid cells were not identified in the peripheral nerves.

**Conclusions:** The distribution and morphology of the globose macrophages in the central nervous system is consistent with globoid cell leukodystrophy, an autosomal recessive lysosomal storage disease. This report documents this condition in three related puppies.

#### **D-075: SUBEPENDYMAL GIANT CELL ASTROCYTOMA IN A CAT: IMMUNOHISTOCHEMICAL CHARACTERISTICS**

Esther Crouch, Mark Troxel, Andrew Miller

**Background:** Subependymal giant cell astrocytoma (SEGA) is the most common central nervous system neoplasm seen in humans with tuberous sclerosis. To date, there is a single report of a SEGA in a domestic cat; however, assessment of immunohistochemical features was limited.

**Objective:** Our objective is to perform a more complete immunohistochemical analysis of feline SEGA.

**Methods:** Gross examination of the fixed brain was performed with representative sections embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. Immunohistochemical staining with rabbit polyclonal antibodies to S100, glial fibrillary acidic protein (GFAP), SOX2, MAP2, synaptophysin, NeuN, and neurofilament was investigated.

**Results:** Arising immediately subjacent to the ependyma and elevating it such that the lumen of the lateral ventricle was markedly narrowed is a well-demarcated, round, unencapsulated neoplasm composed of interlacing streams and bundles of neoplastic astrocytes that have variable morphology. Neoplastic cells were often giant with abundant eosinophilic cytoplasm (gemistocytes) with eccentric nuclei, open chromatin, and a single prominent nucleolus. Immunohistochemistry for S100 illustrated diffuse cytoplasmic immunoreactivity within the neoplastic cells and near diffuse GFAP cytoplasmic immunoreactivity. SOX2 immunohistochemistry revealed intranuclear immunoreactivity in approximately 75% the neoplastic cells. Less than 50% of the neoplastic cells had cytoplasmic immunoreactivity for MAP2, less than 25% had cytoplasmic immunoreactivity for synaptophysin, and intranuclear immunoreactivity for NeuN was rare (less than 1%). Neurofilament lacked immunoreactivity.

**Conclusions:** These histologic and immunohistochemical expression patterns of SOX2, MAP2, and NeuN mirror that seen in human SEGAs and provides further comparative information for this uncommon feline neoplasm.

#### **D-076: SCHWANNOSIS IN THREE FOALS AND A CALF**

Ileana Miranda, Kyle Taylor, William Castleman, Brian Summers, Andrew Miller

**Background:** Conventionally, oligodendrocytes and Schwann cells populate the CNS and PNS respectively. Ectopic intramedullary nests of Schwann cells are found in human Schwannosis, historically associated with neurofibromatosis type 2, but also recognized following some traumatic spinal cord injuries.

**Objective:** To investigate four suspected cases of Schwannosis in domestic animals.

**Methods:** We studied three foals and one calf, 5- to 11-weeks-old, with progressive neurological signs from birth. Histologic examination and immunohistochemistry, including the Schwann cell markers protein zero (P0) and periaxin, were used.

**Results:** In all animals, at multiple levels of the spinal cord, the primary histologic feature was bilateral plaques of proliferative spindle cells, which predominantly affected peripheral white matter adjacent to dorsal and ventral nerve roots, multifocally surrounded blood vessels, and variably extending to the gray matter. In two cases, haphazardly oriented neural tissue also formed a mass effect that compressed the spinal cord leading to syringohydromyelia. The proliferating cells had strong intracytoplasmic immunoreactivity for P0 and periaxin, and both highlighted the formation of PNS myelin within the spinal cord.

**Conclusions:** Lesion immunoreactivity, morphology, and distribution – proximity to CNS-PNS junctions – closely resembles human Schwannosis. Neonatal presentation suggests a congenital malformation with spontaneous invasion of the CNS by PNS-derived Schwann cells.

#### **D-077: CEREBRAL PLAQUES AND NEUROFIBRILLARY TANGLES IN A WOLVERINE (*GULO GULO*)**

Kendall Langsten, Anibal Armien, Tim O'Brien

A sixteen-year-old, 10 kg, intact male wolverine (*Gulo gulo*) housed at the Minnesota Zoo was euthanized due to increasing azotemia, urine dribbling, and a hunched posture. Gross necropsy findings included an 8 cm diameter by 1 cm thick, well-demarcated, non-encapsulated, white to red, firm mass expanding the mucosa of the urinary bladder. Histologic evaluation of all major organs revealed the pathoetiology of the mass was a urothelial carcinoma. Additionally, the cerebral gray matter was multifocally, randomly, and asymmetrically expanded by pale amphophilic, roughly circular accumulations of congophilic and argyrophilic material. Via electron microscopy, this was characterized as approximately 8-15 nm diameter, nonbranching, erratically arranged, extracellular material. Large, argyrophilic neurofibrillary tangles expanded randomly spaced neuronal bodies. Immunohistochemistry for  $\alpha\beta$ -amyloid and hyperphosphorylated tau are pending.  $\text{A}\beta$ -amyloid neuronal plaques in the extracellular matrix and neurofibrillary tangles composed of abnormally phosphorylated tau protein are characteristic histopathological findings in Alzheimer's and Alzheimer's-like disease.

One other case report of a wolverine with Alzheimer's-like disease has been reported, that animal also was housed at the Minnesota Zoo.

#### **D-078: INTRAOCULAR NEURAL HETEROTOPIA IN A MOROCCAN UROMASTYX**

Marta Mainenti, Chad Clancy, Arnaud Van Wettere

**Background:** Neural heterotopia is a rare developmental anomaly consisting of neural tissue in aberrant locations. In humans, heterotopic neural tissue most frequently forms masses in the scalp, nose, lip, nasopharynx, oropharynx, tongue, ear, and orbit. In animals, neural heterotopia has been reported twice in the skin of the frontal head and pharynx of kittens.

**Objectives:** To report a case of intraocular neural heterotopia in a Moroccan Uromastyx.

**Methods:** A 14.3 g, juvenile, female, Moroccan Uromastyx (*Uromastyx acanthinurus nigriventris*) that died unexpectedly after brief rehoming was necropsied.

**Results:** Necropsy revealed minimal intra-coelomic fat stores and small numbers of intestinal nematodes. Eye cross sections revealed one, 4 x 5 mm, grey to white, well-demarcated, expansile, soft mass in the vitreous chamber in each eye. Histologically, both intraocular masses were composed of neuropil with rare glial cells and capillaries, and were contiguous with the optic nerve. No mitotic activity, necrosis, atypia, or increased vascularity were identified. This uromastyx also had metastatic skeletal mineralization.

**Conclusions:** Given the neuropil present in the eyes was well differentiated, a diagnosis of intraocular neural heterotopia was made. Death was attributed to a combination of emaciation and potential vitamin D toxicity. Vision impairment due to the space-occupying, intraocular collection of neuropil is suspected but could not be clinically verified. To the authors' knowledge, this is the first case of intraocular neural heterotopia reported in veterinary medicine.

#### **D-079: ANGIOCENTRIC ASTROCYTOMA IN A CAT**

Brittany McHale, Anibal Armién, Daniel Rissi

**Background:** Gliomas are one of the most common primary central nervous system neoplasms of dogs and cats, but atypical glioma subtypes are rare and the diagnosis is challenging.

**Objective:** To describe an angiocentric astrocytoma in a 15-year-old female spayed domestic shorthair cat that was euthanized after a 3-month history of therapy-resistant seizures.

**Results:** Gross anatomic changes consisted of swelling and hemorrhage of the rostral leptomeninges over the olfactory bulbs that extended into the rostral neuroparenchyma. Histologically, dense aggregates of polygonal to elongate atypical neoplastic cells were arranged along perivascular spaces in the aforementioned areas. Neoplastic cells

concentrically oriented around blood vessels, and had moderate amount of eosinophilic, homogeneous or vacuolated cytoplasm, and round to elongate nuclei with dense or finely stippled chromatin and 1-2 nucleoli. There were two mitoses in ten 400x fields. Neoplastic cells were immunoreactive for glial fibrillary acidic protein, S100 protein, and vimentin. Ultrastructurally, round to elongate neoplastic cells expanded and occupied the spaces between the vascular basement membrane and the *glia limitans*. Cells emitted long cytoplasmic processes containing sparse to dense aggregations of intermediary filaments. Nuclei had small, delicate aggregations of marginal and central heterochromatin. Tight junctions were present connecting the plasma membrane of neighboring cells.

**Conclusions:** The cell morphology, immunohistochemistry, and ultrastructural findings were consistent with an astrocytoma; the exclusive perivascular arrangement of neoplastic cells with no parenchymal mass formation warrants the diagnosis of angiocentric astrocytoma.

#### **D-080: MULTIFOCAL CEREBRAL CORTICAL DYSPLASIA IN A PET PIG WITH ASSOCIATED SEIZURE-LIKE ACTIVITY**

Ethan Biswell, Abigail Durkes, Kaitlin Mielnicki, Janice Kritchevsky

A 7-month-old Vietnamese Potbellied barrow was euthanized after a two month history of increasing seizure-like activity. These episodes included head thrashing, aggressive behavioral change, vision loss, and head tremors. Treatments included antibiotics, steroids, thiamine, and phenobarbital with no improvements. Phenobarbital was discontinued due to vomiting. Bloodwork was within normal limits. An MRI showed symmetric lesions within the cerebral cortex, thalamus, and putamen. Gross examination of fresh tissues revealed no diagnostic lesions. Variation of cortical gray matter thickness was observed after fixation. Histopathologic evaluation revealed multifocal, laminar cortical, thalamic, and putamen dysplasia with mineralization and marginal glioneuronal heterotopia. All other tissues were histologically normal. Special stains (Von Kossa, Prussian blue, and Luxol fast blue with cresyl violet) were performed. Von Kossa was positive for calcium salts in areas that directly match with the reported mineralization on H&E indicating dystrophic calcification (suspect secondary to neuronal necrosis). Prussian blue was negative for iron deposits. Luxol fast blue with cresyl violet clearly showed neurons within cortical white matter (especially in areas where there was loss of distinction of the gray/white matter junction). The cause of clinical illness was attributed to the abnormalities in the brain. The lesions in the brain are suggestive of a chronic progressive congenital malformation that is most consistent with multi-focal cortical dysplasia described in humans. To our knowledge, multi-focal cortical dysplasia has not been reported in pigs. If these lesions are pathophysiologically the same as in humans, surgical removal of dysplastic areas can be curative.

#### **D-081: PARASTRONGYLUS CANTONENSIS CENTRAL NERVOUS SYSTEM INFECTION IN TWO CALLITRICHIDS IN TEXAS**

Erin Edwards, Barbara Lewis, Joe Flanagan

In the spring of 2018, a captive, 8-year-old, male, pied tamarin (*Saguinus bicolor*) was examined due to a sudden onset of seizures. During hospitalization over the next 3 days, the tamarin developed hind limb paralysis and was therefore euthanized. Examination of the brain revealed a moderate eosinophilic meningitis and myelitis with multiple cross sections of metastrongyle larval parasites within the meninges of the cerebrum and cerebellum. Based on the morphologic features of parasites that were extracted from the formalin-fixed meninges, these parasites were identified as *Parastrongylus cantonensis*, previously known as *Angiostrongylus cantonensis*. Additionally, a similar parasite was found within a large pulmonary vessel. The lung is the final site of maturation and reproduction in rodent definitive hosts, but in aberrant hosts such as non-human primates, migration of the parasite typically ceases in the nervous system. In 2015, a Goeldi's monkey (*Callimico goeldii*) from the same institution was diagnosed with a necrotizing and eosinophilic meningoencephalitis and had similar though smaller intralesional larval parasites embedded in the cerebral parenchyma. Though no further work-up was pursued in that case, the parasite is speculated to be *P. cantonensis* as well. Aberrant migration of *P. cantonensis* has been described in the nervous system of several non-human primates across the Gulf Coast. These cases represent the first report of this parasite in non-human primates in Texas.

#### **D-082: AN OUTBREAK OF LEPTOSPIRAL ABORTION AND RENAL DISEASE IN ALPACAS (VICUGNA PACOS) IN NEW ZEALAND**

Fernanda Castillo-Alcala, Susan Brown, Cristin Dwyer, Danielle Aberdein, Alastair Johnstone, Keren Dittmer, Cristina Gans, Sreekumari Rajeev

An outbreak of abortion in breeding females and acute renal disease in juvenile crias occurred in a herd of 70 alpacas in New Zealand. Abortions occurred mid to late gestation, and affected 78% of the pregnant females due to unpack in the spring season (11/14). Concurrently, acute renal disease was diagnosed in 10% of the juvenile crias (2/20), with a 50% mortality rate. Neither placental nor fetal gross lesions were present in all cases examined (n=11). All abortions had evidence of neutrophilic placentitis; two fetuses had mild myocarditis/epicarditis. Postmortem examination of the juvenile cria revealed multifocal mucosal ulceration of C1 and C2. Microscopically, there was acute renal tubular necrosis with intralesional argyrophilic spirochetal organisms, and ulcerative gastritis. Microbiological investigations ruled out common causes of abortion in camelids including *Toxoplasma gondii*, *Neospora caninum*, *Bovine viral diarrhea virus*, coronavirus, herpesvirus, *Campylobacter* spp. and *Mycoplasma* spp. Two females that aborted had paired rising titres for *Leptospira pomona*. Additionally, 23 breeding females from the same herd, and the cria that recovered from acute renal disease, had high titres for *L. pomona* on microscopic agglutination test (>1:3200). *Leptospira* DNA was detected in four placentas using real-time PCR. Leptospiral infection is recognized as a common cause of abortion and renal disease in camelids; however, the clinical signs, lesion distribution and morphology are infrequently described. This is the first report confirming leptospiral abortion and renal disease in alpacas in New Zealand.

### **D-083: COLOR DOPPLER, ULTRASOUND, AND PATHOLOGIC FEATURES OF SPONTANEOUS LEYDIG CELL CARCINOMA OF TESTIS IN BALB/C CMEDC MICE**

Sofia Sacco, Eduardo Belotti, Judith Bertona, Facundo Salinas, Natalia Salvetti, Hugo Ortega

**Background:** Carcinomas of the testis occur spontaneously in Balb/c mice. The color doppler sonographic appearance of Leydig cell carcinoma (LCC) has not been described in mice.

**Objective:** To describe the ultrasound, color Doppler and pathologic features of spontaneous LCC of the testis in Balb/c CMedC mice.

**Methods:** Two, 11 and 12-month-old, respectively, Balb/c CMedC male mice with a history of aggression, anorexia, depression and ascites were submitted for diagnostic work up. The testicles were enlarged, reddish and bluish; they were examined with a color Doppler ultrasound Z6Vet. Necropsies were performed and selected tissues were collected for histopathology.

**Results:** Ultrasound showed a large, multinodular, oval mass of heterogeneous echotexture in one testicle of each mouse. Color doppler revealed central and peripheral, intense hyper-vascularization. Grossly, the tumors were unilateral white to yellowish nodules (10-13 mm diameter) with multifocal cystic cavities and hemorrhagic areas. Histology showed a unencapsulated, multilobulated and densely-cellular neoplasm. This mass was composed of sheets of polyhedral cells with eosinophilic, granular or vacuolated cytoplasm. The cells were supported by a fine fibrovascular stroma. A central round nucleus with stippled chromatin and 1-3 magenta nucleoli was present. Marked anisocytosis and anisokaryosis was also observed. Mitotic rate was ~ 1 per 10 HPF. Metastases were observed in the testicular vein and epididymis of one mouse and in the liver of the other.

**Conclusion:** This report confirms that testicular ultrasound is very useful for the diagnosis of tumors and color doppler can be used to detect areas of angiogenesis in testicular tumors.

### **D-084: COXIELLA BURNETII ABORTIONS IN A EWE HERD**

Jessica Hanlon, José Ramos-Vara, Samuel Yingst, Kenitra Hammac

Two feti from a 4-year-old ewe were submitted to the Animal Disease Diagnostic Laboratory. Several ewes in this herd aborted or delivered stillborn or weak lambs; the ewes were clinically healthy. The gross lesions were confined to the placenta and included multifocal to coalescing, pale tan, raised plaques adhered to the cotyledons, intercotyledonary areas, and umbilicus. Histologically, the cytoplasm of the trophoblasts contained numerous, basophilic coccobacilli. Additionally, chorionic villi were necrotic with areas of mineralization. Many degenerate neutrophils, fewer macrophages, and fibrin infiltrated the stroma of the cotyledon and intercotyledonary areas. Vessel walls were infiltrated and surrounded by similar leukocytes and fibrin. A molecular abortion panel was positive for *Coxiella burnetii* via PCR. Seventeen of the

twenty-five ewes that had lambled within thirty days of the submitted abortion were positive for *Coxiella burnetii* via PCR of vaginal swabs. *Coxiella burnetii* is a gram-negative, zoonotic, obligate intracellular bacterium found in many countries. It causes Q fever in humans and coxiellosis in animals. The most common routes of infection are inhalation or ingestion. When airborne, these organisms can travel over a mile; therefore, humans can become infected without direct exposure to infected animals. Identification and proper reporting of *Coxiella burnetii* infections and rapid implementation of control/management strategies is required to limit the spread of the organism and prevent/decrease human infection.

#### **D-085: CLITORAL CARCINOMA IN DOGS: A RARE OCCURRENCE**

Adam Stern, Mitch Robbins

**Background:** Clitoral carcinoma is a rarely reported neoplasm in the dog. Dogs with this neoplasm have been reported to have clinical signs related to lower urinary tract disease or in some instances, the mass is found incidentally. Approximately 50% of dogs with reported cases of clitoral carcinoma have concurrent hypercalcemia.

**Objective:** This retrospective study aims to characterize clinical and histopathologic features of clitoral carcinoma in two dogs.

**Methods:** Biopsies from the clitoris were submitted between 2010 and 2018. Tissues were processed routinely for histopathological examination and stained with hematoxylin and eosin, Immunohistochemical stains (cytokeratin AE1/AE3, chromogranin A, synaptophysin) were performed on tissue from one case.

**Results:** Both dogs were spayed and greater than 11-years-old. Each presented with a mass arising from the clitoris. Calcium levels were within the normal reference range for both dogs. Histologically neoplastic cells were arranged in sheets, acini, trabeculae, and/or few rosettes. Neoplastic cells from one case were positive for cytokeratin AE1/AE3 and negative for synaptophysin and chromogranin A.

**Conclusion:** Histopathologic features of clitoral carcinoma are similar to that of adenocarcinoma of the apocrine gland of the anal sac. In these dogs, none had blood work to support hypercalcemia of malignancy.

#### **D-086: AN OUTBREAK OF FATAL BORDETELLA BRONCHISEPTICA BRONCHOPNEUMONIA IN PUPPIES**

James Chambers, Isao Matsumoto, Tomoyuki Shibahara, Makoto Haritani, Hiroyuki Nakayama, Kazuyuki Uchida

**Background:** A total of 22 newborn puppies exhibited acute respiratory symptoms and died during one month at a breeding facility. The puppies were from 4 different litters and were not given colostrum for different reasons.

**Objective:** Our objective was to identify the etiologic agent of the outbreak and describe the pathological findings of the disease. The association between colostrum feeding and the fatal infectious disease is discussed.

**Methods:** Necropsy was performed on 4 of the puppies, and the visceral organs were histopathologically examined. Immunohistochemistry, electronmicroscopy and RT-PCR were performed on lung samples in order to detect etiologic agent.

**Results:** At necropsy, the lungs were firm and mottled dark-red, consistent with severe bronchopneumonia. Histopathologically, neutrophils and macrophages severely infiltrated the bronchi and alveoli, and Gram-negative coccobacilli were diffusely attached to the cilia of bronchial epithelium. Immunohistochemistry for *Bordetella bronchiseptica* antigen revealed positive signals of the bacterial agents. On scanning electron microscopy and transmission electron microscopy, numerous coccobacilli were observed attached to the cilia of the bronchial epithelium. Real-time PCR for pathogens of canine infectious respiratory disease detected *B. bronchiseptica* gene from the affected lung tissue.

**Conclusions:** The 4 puppies were diagnosed with fatal *B. bronchiseptica* bronchopneumonia. It is likely that the outbreak at the breeding facility was associated with not feeding the puppies colostrum. The present study demonstrates the pathological findings of fatal *B. bronchiseptica* bronchopneumonia in dogs and the importance of feeding colostrum for prevention of this infectious disease in puppies.

#### **D-087: PULMONARY SILICOSIS IN TWO ADULT ROCK HYRAXES (*PROCAVIA CAPENSIS*)**

Bianca Pfisterer, Robert Donnell, Anthony Ashley, Kim Newkirk

Two rock hyraxes, *Procavia capensis*, from the Chattanooga Zoo were submitted separately for necropsy at the University of Tennessee Veterinary Medical Center. A 4 year-old female hyrax died without premonitory signs and at necropsy had necrohemorrhagic enteritis from which *Clostridium perfringens* and *Escherichia coli* were cultured. A 10 year-old male hyrax was euthanized due to severe chronic lymphocytic tubulointerstitial nephritis and tooth root feed impactions with severe gingivitis and periodontitis. Microscopically, the lungs of both hyraxes had moderate, multifocal to coalescing aggregates (<1mm diameter) of epithelioid macrophages with clear, birefringent, acicular intracellular crystals suggestive of silica. The hyraxes had been housed together on commercially bought play sand composed of 99 to 99.5% crystalline silica. The inhalation of crystalline silicates can induce granulomatous and fibrotic changes within the lungs, resulting in pulmonary silicosis, which is a form of pneumoconiosis. The microscopic findings in these hyraxes, in addition to the history of exposure to crystalline silica, are consistent with pulmonary silicosis. Although neither hyrax had respiratory distress or dyspnea, the pulmonary silicosis may have contributed to the decline of both animals.

#### **D-088: IDIOPATHIC PROGRESSIVE FIBROSING LUNG DISEASE IN A DOG**

Tatiane Terumi Negrão Watanabe, Andrea Dedeaux, Fabio Del Piero

**Background:** Progressive fibrosing lung diseases are unfrequently described in humans, rodents, cat, horses, donkeys, and dogs. They occur during adulthood and

have a progressive clinical course. In horses, equine multinodular pulmonary fibrosis (EMPF) is associated with the gammaherpesvirus equine herpesvirus 5 (EHV-5).

**Case report:** A 6-year-old male, neutered, 58.6 kg Boxer dog, presented with 2-month history of respiratory distress, coughing, and anemia followed by sudden lethargy and anorexia. The owners elected humane euthanasia and post-mortem examination was performed.

**Results:** The lung lobes were scarred by a network of depressed grayish firm areas. Histologically, the alveolar septa were multifocally and severely thickened by reactive fibroblasts and fibrous connective tissue randomly distributed throughout the parenchyma. The alveolar spaces were either atelectatic or expanded by large numbers of foamy alveolar macrophages and lined by hypertrophic and hyperplastic type II pneumocytes with occasional cytomegaly and syncytial cells. The interstitium was infiltrated by low numbers of lymphocytes, plasma cells, and rare macrophages. The kidneys had multifocal extensive cortical areas of acute ischemic coagulative renal tubular degeneration and necrosis. The liver had centrilobular hepatocellular degeneration and necrosis. No gammaherpesviruses were detected via PCR in the affected lung. No other agents were observed via bacterial culture and histochemistry. There was no history of exposure to toxic substances.

**Conclusions:** Here we describe the clinical, pathologic and molecular findings of a severe form of idiopathic pulmonary fibrosis in a dog.

## **D-089: NASAL SEROMUCINOUS HAMARTOMA IN A HORSE**

FABIO ROSA, Joseph Newton, Jey Koehler

**Background:** A 12-year-old, castrated male, American Quarter Horse was presented for necropsy with a history of bilateral nasal discharge and swelling of the frontal bone. Computed tomography showed a soft tissue mass occupying the middle and caudal aspect of the nasal cavity completely obliterating the paranasal sinuses. The mass extended into the nasal choanae and nasopharynx.

**Objective:** Our objective was to diagnose the mass.

**Methods:** Necropsy was performed and histopathologic sections of the nasal mass were stained with H&E and immunohistochemically for Ki-67.

**Results:** At necropsy, there was a protuberance of the frontal bone; a large multilobulated, firm and gritty mass and a yellow, mucoid exudate in both the right and left paranasal sinuses. A portion of the mass extended through the distal aspect of the hard palate and extended into the soft palate and into the pharynx. There were multifocal areas of osteolysis of the frontal bone, dorsal maxilla and the cribriform plate. Histologically, the mass was lined by respiratory epithelium, formed polypoid projections and was composed of numerous, tortuous seromucinous glands widely separated by a fibrovascular to mucinous stroma. Cellular atypia, cellular and nuclear pleomorphism and mitotic figures were absent.

**Conclusion:** With immunohistochemistry for Ki-67, neoplastic cells showed no proliferation and the mass was diagnosed as a seromucinous hamartoma. Seromucinous hamartomas are rare benign glandular proliferations that occur in the sinonasal tract and nasopharynx, often forming polyps. A considered differential diagnosis was a low grade adenocarcinoma but the histologic evidence did not support this diagnosis.

**D-090: IMMUNOHISTOCHEMICAL CHARACTERIZATION OF SPONTANEOUS BRANCHIOBLASTOMA IN A KOI CARP (CYPRINUS CARPIO) AND BLUEGILL SUNFISH (LEPOMIS MACROCHIRUS)**

Elizabeth Alloway, Jesse Riker, Heather Shive, Brigid Troan, Emily Christiansen, Gregory Lewbart

Branchioblastomas, neoplasms of primitive branchial cells, have been identified in several species of fish. These neoplasms can be induced experimentally with the administration of N-methyl-N'-nitro-N-nitrosoguanidine and nifurpirinol, but spontaneous neoplasms are rarer, with reports limited to rainbow trout, brown trout, koi carp, and an Indian oil sardine. This case report describes spontaneous branchioblastoma in a koi carp with lymphosarcoma and a Bluegill sunfish (*Lepomis macrochirus*), with comparison of primary and recurrent branchioblastoma in the Bluegill sunfish after surgical resection. In both cases, the branchioblastomas were well-demarcated, multilobulated, soft, pink masses arising from the pseudobranch (koi carp) or first branchial arch (Bluegill sunfish). The masses were composed of three intimately associated neoplastic populations: epithelial trabeculae and cords, cartilaginous islands and lamella-like structures; and sheets of blastemal cells. Immunohistochemistry demonstrated that neoplastic cells with chondrocyte morphology displayed strong nuclear immunoreactivity for sox9, consistent with chondroid differentiation. Both epithelial and chondroid populations displayed strong cytoplasmic immunoreactivity for pancytokeratin, while the blastemal cells were consistently negative for all three immunomarkers. The diagnostic utility of vimentin was limited in this analysis, as no normal or neoplastic tissue displayed consistent immunoreactivity. Interestingly, comparison of the primary and recurrent branchioblastoma from the Bluegill sunfish demonstrated loss of cartilaginous differentiation with concurrent loss of sox9 immunoreactivity in the recurrent tumor; however, epithelial and blastemal components were similar in these specimens. These results describe the first case of spontaneous branchioblastoma with recurrence in a Bluegill Sunfish, and provide the first immunohistochemical characterization of branchioblastoma in fish species.

**D-091: MESOTHELIAL PROLIFERATION MIMICKING NEOPLASTIC DISEASE PROCESS IN A BABOON (PAPIO SPP.)**

Olga Gonzalez, Erin Ball, Shyamesh Kumar, Edward Dick Jr

**Background:** Distinguishing benign versus malignant thoracic mesothelial proliferations is a diagnostic challenge.

**Methods:** A six-year-old male baboon was euthanized due to respiratory distress. Eleven months before, this animal had participated in a hemorrhage study from which

he recovered uneventfully. Standard necropsy was performed; all tissues were processed conventionally. Ancillary histochemical and immunohistochemical staining were performed as per standard protocols.

**Results:** On gross examination, there was marked thickening of the diaphragm with white firm tissue and regional dark red cavitated areas. The visceral pleura of all lung lobes was adhered to the parietal pleura of the thoracic cavity and diaphragm. The abdominal surface of the diaphragm had numerous firm tan nodules that were also noted throughout the serosa of the stomach, omentum, mesentery, and mesocolon. Histopathology revealed a population of spindle cells arranged in streams, bundles, and nodular foci with interspersed collagen deposits and minimal supporting vascular stroma. Some areas resembled mature granulation tissue. Twenty percent of the spindle cells within nodular foci co-labeled with vimentin and pancytokeratin (AE1/AE3), and BRCA1 associated protein-1. The proliferative tissue was negative for mesothelioma markers (calretinin, CK5/6, WT-1, and D2-40/podoplanin).

**Conclusions:** The immunohistochemical profile, cell morphology, and arrangement of the proliferative pleural tissue was most consistent with benign/reactive process of undetermined origin. The etiology for the mesothelial proliferation is uncertain, although post-protocol related physiological hyperplasia cannot be ruled out. This case has a unique gross and histopathological appearance mimicking a neoplastic process.

#### **D-092: TOXIC PNEUMONITIS CAUSED BY WATERPROOFING SPRAY IN A DOG**

Yan-Xiu Lin, Hao-Kai Chang, Shiun-Long Lin, Hue-Ying Chiou, Jiunn-Wang Liao

A 7-year-old, intact male, Dachshund dog was presented for respiratory signs after it stayed in the room where the owner used waterproofing spray containing fluorocarbon polymers. Acute intoxicated signs of tachypnea and respiratory effort were observed in the physical examination. Clinical pathological abnormalities showed neutrophilia. Thoracic radiographic examination showed bilateral interstitial to alveolar pattern. After 6 days, the dog became poor appetite and dyspnea. The dog's condition continued to deteriorate and died next day. Necropsy was performed at the Animal Disease Diagnostic Center, NCHU. Gross findings showed all lung lobes were diffuse red and palpated meaty. On cut surfaces, the airways did not contain fluid or exudate. Cytological examination of stamp smears revealed hyperplastic cuboidal to columnar epithelium, neutrophils and macrophages, as well as spindle-shaped cells. Histopathologic findings included bronchial and bronchiolar dilatation, diffuse desquamation of the bronchiolar epithelial cell layers and alveolar ducts, moderately thickened alveolar septa with hyperplasia of pleomorphic type II pneumocytes and prominent syncytial cells. Moderate fibrosis was found either by Sirius Red stain or Masson's trichrome stain methods. The differential diagnosis included toxic pneumonitis, hypersensitivity pneumonitis and viral pneumonia (canine distemper virus). Waterproofing spray was considered as the most likely acute toxin according to the history. In human, inhalation of waterproofing spray containing fluorocarbon polymers is considered to be strongly associated with acute respiratory damage. Based on history, symptoms, thoracic radiographic findings and pathological examination, final diagnosis was toxic pneumonitis caused by inhalation of waterproofing spray.

#### **D-093: ACHYLA SPP DERMATITIS IN AN AMERICAN ALLIGATOR (ALLIGATOR MISSISSIPPIENSIS)**

Carmen Lau, Laura Bryan, Eric Snook

Oomycetes are water molds in the Kingdom Protista and not considered true fungi due to the structural lack of chitin and ergosterol. Many oomycetes are pathogenic, such as *Pythium* spp, and many aquatic fishes are prone to *Saprolegnia* spp infections, particularly in stressful farming situations. Two juvenile American alligators (*Alligator mississippiensis*) presented for necropsy with white, gelatinous, raised lesions over ulcerated regions of skin on the limbs and tails. The alligators were from a hatchery with age-divided enclosures, and several of the animals within the same enclosure showed similar lesions. Numerous fungal hyphae with nonparallel walls and sparse, non-dichotomous branching were observed histologically by GMS within a severe, multifocal, chronic necroulcerative and granulomatous dermatitis. Although no fungal organisms were able to be amplified by PCR on fresh or formalin fixed tissues, the organism was cultured and sequenced as an *Achylya* spp., a less frequently identified oomycete. Oomycete infection of reptiles and chelonians is rarely reported, and this is the first description of an *Achylya* spp. infection within the class Reptilia.

#### **D-094: IMMERSION FOOT SYNDROME IN 6 EQUIDS EXPOSED TO HURRICANE FLOODWATERS**

Brianne Taylor, Aline Rodrigues-Hoffmann, Angela Arenas-Gamboa

Prolonged exposure to water, known as immersion foot syndrome (IFS) in humans, is a multifactorial phenomenon first described in soldiers during World War I. IFS is characterized by changes in dermal microvasculature and subsequent ischemic necrosis. In this report, we describe the pathologic findings of a condition resembling IFS in equids with prolonged floodwater exposure during Hurricane Harvey. Five horses and one donkey presented for necropsy at Texas A&M University two to four weeks after initial exposure to floods in Texas. At necropsy, all animals had similar dermal changes, including numerous abrasions, clefts, and linear fissures. These lesions were ventral to a sharply-demarcated “water line” that ran along the lateral trunk and became increasingly severe on the distal limbs. In five of the six animals, histologic examination revealed moderate to severe vasculitis, perivascular dermatitis, and coagulative necrosis consistent with ischemia. The severity of histologic lesions progressed from ventral trunk to distal limbs and became more pronounced in cases that presented in the weeks following the hurricane. The pathophysiology of IFS is multifactorial and results from the sympathetic responses in the dermal microvasculature, from initial vasoconstriction to reflex vasodilation, leading to thrombosis and ischemia. This is the first report to characterize a condition similar to immersion foot syndrome in humans following prolonged water exposure.

#### **D-095: SQUAMOUS CELL CARCINOMA ARISING IN CHRONIC EXFOLIATIVE CUTANEOUS LUPUS ERYTHEMATOSUS OF THE GERMAN SHORTHAIRED POINTER**

Wallaya Manatchaiworakul, Annette Petersen, Erica Noland

Squamous cell carcinoma (SCC) arising in forms of chronic cutaneous lupus erythematosus (CCLE) is a recognized rare complication in humans. Similar rare reports have been made in dogs. Possible contributing factors are likely multifactorial and include chronic ultraviolet light exposure, chronic scarring, papillomavirus infection, and long-term immunosuppressive therapy. Here, a 1-year, spayed female German Shorthaired Pointer had clinical signs of multifocal progressive hair loss and scaling in 2008, and was diagnosed with exfoliative cutaneous lupus erythematosus (ECLE) of the German Shorthaired Pointer by histopathologic examination in 2009. In August 2017, this dog presented for a two month history of an alopecic mass on the medial left hind leg, which was diagnosed as SCC. Recurrence occurred in December 2017. At the periphery of both the original and recurrent masses, there were histologic lesions, consistent ECLE. In the original mass, there was a focus of tangled elastin fibers at one lateral margin of the SCC noted on histochemical staining. The majority of neoplastic cells showed strong nuclear immunoreactivity for p53, and basal keratinocytes within the immediately adjacent non-neoplastic epidermis had similar p53 expression. In a retrospective analysis from January 2005 to May 2018, 117 cases were diagnosed at MSU with a form of lupus erythematosus. Of these cases, there were no reports of concurrent squamous cell carcinoma or epithelial dysplasia; however, additional biopsies at later time points were not received. While the association is rare, clinical screening for proliferative epithelial changes in CCLE is warranted.

#### **D-096: CHARACTERIZATION OF CUTANEOUS CORYNEBACTERIUM BOVIS INFECTION IN NSG MICE BY HISTOPATHOLOGY AND LASER CAPTURE MICRODISSECTION**

Lauren Himmel, Austin Southard-Smith, Katherine Shuster, Kenneth Henderson, Kelli Boyd

**Premise:** *Corynebacterium bovis* is a well-characterized cutaneous pathogen of athymic nude mice, in which it is the causative agent of scaly skin disease, but little is documented about its clinicopathologic manifestation in other haired immunocompromised strains. Five NOD SCID gamma (NSG) (NOD.Cg-*Prkdc*<sup>scid</sup>*Il2rg*<sup>tm1Wjl</sup>/SzJ) mice presented with mild facial alopecia, erythema, and scaling. Herein, we document the distinct gross and microscopic pathology of *C. bovis* skin disease in the NSG mouse and demonstrate the utility of laser capture microdissection (LCM) and PCR to identify this pathogen in formalin fixed, paraffin embedded (FFPE) tissue.

**Methods:** A clinical diagnosis of *C. bovis* was achieved by PCR on skin swabs collected at the time of necropsy. Serially sectioned FFPE skin was used for H&E staining, Gram stain, and LCM.

**Results:** Histopathology of the skin lesions was distinct from that previously reported in nude and SCID mice, demonstrating large intracorneal and intrafollicular aggregates of Gram positive bacteria. A presumptive diagnosis of coryneform hyperkeratosis was made. Bacteria isolated from skin sections by LCM were subsequently identified as *C. bovis* by 16S rRNA PCR.

**Conclusions:** Given the ubiquity of *C. bovis* and increasing popularity of severely immunocompromised mice in research, clinical veterinarians and veterinary pathologists should be familiar with its unique presentation in the NSG mouse. Furthermore, we demonstrate the utility of LCM plus PCR to accurately and efficiently identify *C. bovis* in FFPE tissue sections.

**D-097: ACCESSORY TRAGUS: A HUMAN CONGENITAL ANOMALY IN A DOG**

Becky Lee, Alisha Massa, Kyle Taylor

Accessory tragus is a congenital malformation of part of the external ear, reported in humans. Clinically, it is a benign, cutaneous mass located anywhere between the tragus of the ear and the angle of the mouth, along the migratory path of the first branchial arch. We present a striking case of accessory tragus in an otherwise healthy, six-month-old Pitbull with a haired, pedunculated cutaneous mass on its cheek—over the ventral masseter muscle—from birth. Histologically, the mass was a pedunculated, polypoid extension of histologically normal haired skin with a central core of well-differentiated elastic cartilage. To the authors' knowledge, this is the first time this lesion has been described in a non-human species, and a retrospective examination of records at the Washington State Animal Disease Diagnostic Laboratory (WADDL) demonstrates the paucity of similar lesions among myriad cases of the primary differential diagnoses: skin tag or acrochordon and collagenous hamartoma.

**D-098: HYPOTRICHOSIS IN ANGUS CALVES CAUSED BY PRESUMPTIVE BOVINE VIRAL DIARRHEA VIRUS INFECTION IN A UTAH BEEF HERD**

Michael Clayton, Phillip Firth, Gordon Hullinger, Jeffery Hall, Arnaud Van Wettere

Hypotrichosis is a rare lesion associated with in utero bovine viral diarrhea virus (BVDV) infection in calves. We report an outbreak in an Angus beef herd, over two consecutive years, where hypotrichosis was the main gross lesion. Late term abortion or death within 24 hours of parturition occurred in 10 and 50, out of 130 calves in 2016 and 2017, respectively. Two calves (2016) and four calves (2017) submitted for postmortem examination had mild to severe generalized alopecia with sparing of the distal extremities, and gastrointestinal tracts were filled with large amounts of hair. Histologically, there was a reduction in the number of erupting hair shafts in the skin, and hypomyelination in the brain. BVDV was detected by PCR in 1 out of the 6 calves necropsied. BVDV antibody titers, on antemortem serum samples, were detected by virus neutralization in 2 of 3 calves necropsied in 2017, however colostrum had been administered making interpretation difficult. Very high BVDV antibody titers were detected by serum neutralization in 7 out of 7 cows tested in 2016 (BVDV type 1 and 2) and 19 out of 32 cows tested in 2017 (BVDV type 2), with vaccination having been greater than 9 months prior to testing. BVDV antigen was detected in ear notches of 2 out of 28 cows by ELISA in 2017. The cause of neonatal death with hypotrichosis and hypomyelination are presumed to be due to intrauterine BVDV type 2 infection.

**D-099: IDIOPATHIC SKIN FRAGILITY SYNDROME IN A CAT**

Nohemí Vazquez Mota, Gisela Fuentes Mascorro, Miguel Dominguez Martinez, Ramón Leon Zetina

A 4.5 month male Domestic Shorthair cat presented for three months history of tearing and spontaneous skin non-bleeding lacerations, some caused during the clinical management. Despite the considerable improvement in post-treatment body condition, delays in the repair capacity were observed in the lesions and due to multiple lacerations in different parts of the body, euthanasia was chosen. The post mortem examination confirmed skin fragility, with skin tearing during slight tension, without hyperelasticity. In the histopathological examination, epidermal and dermal atrophy was found as well as lack of integrity in the epidermo-dermal junction and absence of lesions in organs such as pancreas, liver, adrenal and pituitary. The morphological evidence was consistent with Idiopathic Feline Cutaneous Fragility Syndrome (SFSFi). The SFSFi is a low casuistry disease, without a case report in Mexico and scarce information on its clinical-pathological behavior, thus, this report is transcendental as the first in the country.

#### **D-101: CORYNEFORM HYPERKERATOSIS IN NOG MICE**

Yan-Xiu Lin, Yun-Chieh Tuan, Fang-Yi Tsai, Ying-Chen Wu, Ji-Hang Yin, Jiuun-Wang Liao

Male NOG mice, 14 weeks old, showed multiple approximately  $0.1 \times 0.2 \times 0.1$  cm pale tan to brown scale-like crusts on the shaved area at the dorsal aspect of the skin. The morbidity was 9.8% (5/51). Histological findings in the affected haired skin was multifocal, moderate chronic hyperkeratosis with moderate acanthosis. In addition, basophilic bacterial clumps were found in the keratin layer. Impression smear from the scrapping through the lesional areas stained with Diff-Quik revealed gram positive, clubbed shaped and V shaped arrangement rod bacteria, which was identified through 16S rRNA gene sequencing and had 98% similarity with *Corynebacterium mastitidis*. Furthermore, same bacteria were cultured from the hair removed from the razor. Differential diagnoses in this case including scaly skin disease, etiology consistent with *Corynebacterium bovis*, and particularly frequent observed in the experimental mice, bacterial infection such as *Staphylococcus xylosus*, *Proteus* and opportunistic bacteria, and low ambient humidity. However, *Corynebacterium mastitidis* was cultured predominately in this case and the environment the NOG mice habituated were reported adequately. Therefore, Coryneform hyperkeratosis in NOG mice was made in this case.

#### **D-102: CASE REPORT: GASTRIC ADENOCARCINOMA IN CD-1 MOUSE**

Marie Bockenstedt, Alok Sharma

A control mouse used for a carcinogenicity study presented on day 442 of the dosing phase for debilitating condition (pallor, hunched posture, and discolored black feces). Upon necropsy, a large spleen and duodenal intussusception were noted. Microscopic examination revealed a gastric mass at the pyloric junction extending into the duodenum. The glandular stomach had a focal polypoid mass composed of two cell populations; cuboidal to columnar cells forming tubules and solid nests of round to oval neoplastic cells with indistinct cell borders. Intermingled with the neoplastic cells were multiple foci of immature bone. The mass was consistent with a gastric adenocarcinoma with bone formation. Differential diagnoses for the finding included osteosarcoma, gastric adenoma, gastric mucosal hyperplasia, and a neuroendocrine cell tumor.

Neoplasms of the glandular stomach are uncommon in mice and as per published reports incidence of adenomas or adenocarcinomas in the stomach of CD1 mice is 0-3%. The incidence of gastric adenocarcinoma on this study was 0.3% in males and females. A review of the internal historical control database yielded no incidence of gastric adenomas or adenocarcinomas in CD1 mice. Gastric adenocarcinoma with bone formation observed in our study is a rare spontaneous neoplasm affecting CD-1 mice with no published reports.

#### **D-103: ALDICARB POISONING IN 2 DOGS**

Guillermo Rimoldi, Douglas Smith, John Buchweitz

Two dogs suspected malicious poisoning, were submitted for necropsy to Clemson Veterinary Diagnostic Center. First case, February 2018, was a one-year-old male Labrador mix, with history of acute onset of vomiting, diarrhea, and lethargy, followed by death in less than one hour. Another seven dogs were similarly affected, but were not submitted for necropsy. The second case, April 2018, was an 11-week-old male boxer cross with history of going from active play to seizing, foaming from the mouth, and death less than one hour later. Gross and histological findings were similar in both dogs and consisted of multiple, subcapsular pancreatic hemorrhages, extending into and expanding interlobular septa, without necrosis. Lungs were diffusely, moderately congested and edematous. Stomach contents toxicological screen detected aldicarb in each dog (GCMS). Additionally, acetyl-cholinesterase activity was suppressed in the first dog's brain, 0.6  $\mu\text{mol/g/min}$  (ref interval 2.88-5.1), consistent with carbamates exposure. Aldicarb toxicosis cases describe pancreatic hemorrhages as in these two dogs, the mechanism(s) responsible for this change are unknown. Aldicarb is a carbamate pesticide extensively used on crops. Outside the USA, it is widely available and commonly reported as being used to quickly kill guard dogs to facilitate illegal entry into properties. In the USA, due to its high toxicity and environmental impacts, aldicarb was removed from widespread distribution and is only used in controlled applications. Sudden death, or a clinical history as in these two cases, together with the presence of pancreatic hemorrhages, should alert veterinary pathologists to aldicarb toxicosis as a potential etiology.

#### **D-104: RENAL TUBULAR DYSGENESIS WITH CONCURRENT OSTEOGENESIS IMPERFECTA, DENTINOGENESIS IMPERFECTA, AND GROWTH ARREST IN A YOUNG DOG**

William Sills, Roy Pool, Katie Kelly

**Background:** An 11-month old, male Staffordshire Bull Terrier dog died after a long history of growth arrest, joint laxity, recurrent respiratory infections, and recurrent juvenile cellulitis.

**Objective:** To describe a case of concurrent renal tubular dysgenesis (RTD) and skeletal and dental malformations in a young dog.

**Methods:** A full necropsy was conducted, and all major tissues, including several sections of long bones and mandible, were examined histologically.

**Results:** Gross evaluation revealed small stature, low body weight (4.2 kg), and severely shortened long bones of all limbs. The teeth were disproportionately large and soft with dull enamel. The frontal and maxillary sinuses were markedly narrow to absent with deformed nasal turbinates. Long bones exhibited severe cortical osteopenia and widespread depletion of cancellous bone, consistent with osteogenesis imperfecta. Teeth lacked dentin in the pulp cavity (dentinogenesis imperfecta). There was renal tubular dysgenesis (RTD) with nearly diffuse malformation of proximal tubules, characterized by narrow, diminutive hypercellular tubules with narrow to indiscernible lumens and thickened basement membranes which were embedded within abundant fibrous connective tissue and interspersed between mature glomeruli. Other findings included severe pulmonary and widespread vascular mineralization.

**Conclusions:** RTD is a rare human clinical fetal disorder characterized by the absence or poor development of proximal tubules. RTD is rarely reported in veterinary species but this case demonstrates that this rare renal malformation can occur with complex congenital skeletal disease; there is no clear shared association between these renal and skeletal malformations.

#### **D-105: GLOMERULAR ULTRASTRUCTURAL FINDINGS IN PIGS WITH PORCINE DERMATOPATHY AND NEPHROPATHY SYNDROME**

Christopher Siepker, Carrie Schmidt, Cathy Brown

**Background:** Porcine circovirus is a small DNA virus which is most commonly associated with postweaning multisystemic wasting syndrome (PMWS) in piglets. Porcine circovirus type 2 (PCV2) infection in commercial swine is a complex viral infection which most commonly results in severe lymphoid depletion, clinically manifesting as poor body condition and enlarged lymph nodes, causing economic loss worldwide. PCV2 infection has also been associated with Porcine dermatopathy and nephropathy syndrome (PDNS), manifested grossly as dark red to purple discolorations on the skin and kidneys. The light microscopic findings of PDNS are those of an exudative glomerular disease, with erythrocytes, fibrin, and inflammatory cells within Bowman's space. While this unique manifestation of porcine circovirus associated disease (PCVAD) is thought to represent immune-mediated sequela to viral infection, the ultrastructural glomerular lesions have not been previously described.

**Objective:** Evaluate glomeruli in swine with PDNS by ultrastructure for the deposition of immune complexes.

**Methods:** Swine tissues were evaluated by immunohistochemistry (IHC) to confirm circovirus infection and transmission electron microscopy (TEM) was utilized to evaluate the affected glomeruli ultrastructurally.

**Results:** Glomeruli exhibited podocyte effacement and Bowman's space contained abundant fibrin, neutrophils, and erythrocytes. Focal rupture of the glomerular basement membrane was present. Rare mesangial electron dense deposits in occasional glomeruli were consistent with nonspecific trapping.

**Conclusions:** Ultrastructural findings differ from those seen with primary endothelial injury (thrombotic microangiopathy) or immune-complex glomerulonephritis (membranoproliferative glomerulonephritis). The lesions are similar to those described in people with pauci-immune crescentic glomerulonephritis, an Antineutrophil Cytoplasmic Antibody (ANCA)-associated renal vasculitis.

**D-106: CILIATE INFECTION ASSOCIATED WITH CUTANEOUS ULCERATION IN SEA PENS (ORDER: PENNATULACEA)**

Emily Corbin, Michael Garner, Elise LaDouceur

**Background:** Protozoal diseases are uncommonly described in animals from the class anthozoa, which includes corals, anemones, and sea pens. This study describes disease presentations and lesions associated with ciliate infections in sea pens (order: pennatulacea). Although recently associated with significant disease in coral, disease associated with ciliate infection has not been previously described in sea pens.

**Methods:** Archives of Northwest ZooPath were searched for sea pens with ciliate infections. Histology was reviewed, and scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were performed.

**Results:** Four sea pens were identified with ciliate infections. Clinical observations included dull coloration, cutaneous erosion, cutaneous sloughs, and lateral recumbency. In each case, ciliates were seen in cytologic examination of skin scrapings. One animal was euthanized, two were found dead, and one was biopsied and subsequently lost to follow up. Histologically, ciliate infection was consistently associated with cutaneous ulceration, necrosis, and infiltration of amoebocytes. Ultrastructurally, SEM of protozoa revealed dense cilia distributed over the entire body. TEM revealed anatomic detail of the cilia, alveoli, kinetome, (i.e. organelle system associated with cilia), and macronucleus.

**Conclusions:** As ciliates were consistently associated with ulcers in this series, it is suspected that this infection is a substantial contributor to cutaneous ulceration. It is unclear if the ciliates are the cause of ulceration and subsequent mortality, or if ciliates secondarily colonize ulcers that are caused by other factors. This is the first description of ciliate-associated disease in sea pens.

**D-107: THYMOMA IN A COMMON BOTTLENOSE DOLPHIN (TURSIOPS TRUNCATUS) RESEMBLING HUMAN TYPE B3 AND STAGE II THYMOMA**

Wen-Ta Li, Chieh Lo, Chian-Ren Jeng

**Background:** Thymomas are neoplasms derived from thymic epithelial cells with benign lymphoid proliferation. Although thymomas have been reported in a variety of wildlife species, no cases of thymoma have been reported in either captive or free-ranging cetaceans.

**Case presentation:** An adult female bottlenose dolphin was stranded and found dead on the east coast of Taiwan. Necropsy and tissue sample collection for histopathology were performed.

**Results:** There were several round-shaped wounds (caused by cookiecutter sharks) on the left-dorsal aspect of body trunk. A fixed, well-encapsulated, smooth bosselated, 5 x 4 x 3 cm mass was found in the middle mediastinal region, and the cut surface showed variable-sized, jigsaw puzzle-like, white to tan nodules demarcated by white sclerotic septa. The mass comprised lobules of polygonal neoplastic cells associated with scattering aggregates of CD3+ T-lymphocytes and separated by thick fibrotic stroma. Aggregates of neoplastic cells were noted in the capsular area. The neoplastic cells were intensely positive for cytokeratin and P63, focally positive for CK19, and negative for CD5, CD117, vimentin, thyroid transcription factor-1, synaptophysin and chromogranin A.

**Conclusion:** In humans, thymomas are classified into five subtypes (A, AB, B1, B2 and B3) and four stages (I to IV) by the World Health Organization histologic classification and Masaoka-Koga staging system, respectively. Patients with type B3 or stage IV thymoma usually have a relatively worse prognosis. Based on the anatomic location and the histopathologic/immunohistochemical characteristics, the neoplasm was considered a thymoma resembling human type B3 and stage II thymoma.

#### **D-108: PATHOLOGIC FINDINGS IN AN AGED CAPTIVE SOUTHERN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)**

Lisa Mangus, Lauren Peiffer, Ellen Bronson

A 48-year-old, female Southern white rhinoceros (*Ceratotherium simum*) housed at the Maryland Zoo in Baltimore was humanely euthanized due to quality of life concerns including progressive right forelimb lameness that ceased responding to medical management. Full gross and histopathologic examination revealed numerous pathologic processes affecting multiple organ systems. The right adrenal gland was larger than the left due to diffuse expansion of the medulla (cortical to medullary ratio approximately 1:8). Within the medulla were multiple soft tissue masses, identified histologically as a single pheochromocytoma and multiple (three) myelolipomas. The medulla of the left adrenal gland was replaced by a well-demarcated, soft tissue mass that compressed the cortex and was identified histologically as a myelolipoma. Grossly, the uterine body was effaced by multiple, coalescing soft tissue tumors. Histologically, these tumors were composed of variably differentiated spindloid cells admixed with areas of necrosis and hemorrhage (leiomyosarcoma). The kidneys contained hundreds of variably-sized, cavities filled with clear yellow fluid. Histologically, these cavities were lined with cuboidal to attenuated epithelium, consistent with renal cysts. Renal arteries demonstrated appreciable proliferation of the tunica intima with restriction to occlusion of the lumen (arteriosclerosis). Arteries of multiple organs (heart, stomach, lungs, kidneys) were thickened due to intimal and medial hypertrophy and hyperplasia (arteriosclerosis). Chronic osteoarthritis was present in all appendicular joints, most severely affecting both stifles and elbows. This case documents multiple neoplastic and degenerative lesions that have been individually described in aged rhinoceroses and

adds to our understanding of disease processes affecting this infrequently examined species.

#### **D-109: RANAVIRUS: FIRST DESCRIBED CHELONIAN CASES IN ONTARIO, CANADA**

Christina McKenzie, Douglas Campbell, Heindrich Snyman, Tomy Joseph, Christina Davy, Claire Jardine

**Background:** Frog virus 3 (FV3), a ranavirus, causes systemic disease in ectothermic vertebrates such as amphibians, fish and reptiles. It is most commonly found in amphibians and can cause mass mortality events in juveniles. FV3 has also been found to cause disease in a variety of turtle species and has been associated with mass mortality events in box turtles in the United States. Many Ontario turtles are protected species and the wood turtle (*Glyptemys insculpta*) is listed as endangered. Loss of adult turtles can have a devastating impact on populations given their long life span and low fecundity.

**Objectives:** This study sought to describe the pathology associated with the first cases of ranavirus detected in turtles in Ontario.

**Methods:** Specimens were examined microscopically and tested by PCR.

**Results:** The first documentation of FV3 in a turtle in Canada occurred in a free-ranging snapping turtle (*Chelydra serpentina*) collected from southwestern Ontario in 2017. Since then, a wood turtle and two painted turtles (*Chrysemys picta*) have been diagnosed. To the author's knowledge, these are the first reported cases of FV3 in these species. Gross lesions included oral plaques, palpebral edema, conjunctivitis and dermal ulceration. Microscopic lesions included fibrinonecrotizing vasculitis in the conjunctiva, spleen, liver, kidney and lungs as well as necrotizing splenitis. The presence of FV3 was confirmed via PCR in all cases. All specimens tested negative for herpesvirus.

**Conclusions:** FV3 has been found to cause mortality in snapping turtles, wood turtles and painted turtles in Canada.

#### **D-110: ENDOCARDIOSIS IN TETRAS (FAMILY: CHARACIDAE)**

Robert Kim, Andrew Cartoceti, Judy St. Leger, Roy Yanong, Kali Holder, Elise LaDouceur

**Background:** Endocardiosis has been described in zebrafish (*Danio rerio*, family: Cyprinidae), and reported anecdotally in Atlantic salmon (*Salmo salar*, family: Salmonidae), in which the significance of this disease is unknown. This study describes endocardiosis in tetras (family: Characidae) with comparisons to published lesions of endocardiosis in zebrafish.

**Methods:** Necropsy records of tetras from two institutions were searched for cardiac lesions consistent with endocardiosis. Five cases were identified and histology was reviewed. Control tetras (*Paracheirodon innesi*) were also evaluated histologically.

**Results:** Endocardiosis was identified in five tetras (four *Paracheirodon innesi* and one *Hemigrammus rhodostomus*) that were at least 8 months old. Four tetras were found dead, and one was euthanized for coelomic distention. Histologically, proliferative myxomatous matrix expanded multiple cardiac valves (valvular endocardiosis) and/or cardiac wall (mural endocardiosis), and frequently occluded a substantial portion of cardiac chamber lumina in the sections examined. Endocardiosis was considered the cause of death in four tetras due to severity of endocardiosis and lack of identifiable comorbidities. One tetra had concurrent egg binding, and this case had the least severe endocardiosis, which was considered a comorbidity, but not necessarily the cause of death. Retrospective review of tetra submissions from two institutions suggested a prevalence of endocardiosis in tetras of 5.7% and 4.3%, respectively.

**Conclusions:** In zebrafish, endocardiosis lesions ranged in severity, but were uniformly substantially less severe than these lesions in tetras. Endocardiosis is a previously unreported contributor to mortality in tetras, in which this disease can cause nearly complete occlusion of cardiac chambers.

#### **D-111: FIRST DETECTION OF EPIZOOTIC HEMORRHAGIC DISEASE IN WILD WHITE-TAILED DEER IN ONTARIO, CANADA**

Samantha Allen, Jamie Rothenburger, Claire Jardine, Aruna Ambagala, Kathleen Hooper-McGrevy, Mark Ruder, Nicole Nemeth

**Background:** Epizootic hemorrhagic disease virus (EHDV) affects wild and domestic ruminants and has gradually spread northward within the U.S. over the past 30 years. However, outbreaks in more northern regions have become more frequent and in 2017, an EHDV-2 outbreak occurred in white-tailed deer (*Odocoileus virginianus*) across parts of the eastern U.S.

**Objective:** Our objective was to identify and characterize the first cases of EHDV in wild white-tailed deer in east-central Canada.

**Methods:** Field-collected white-tailed deer carcasses underwent full postmortem examination (gross and histopathology) at the Canadian Wildlife Health Cooperative-Ontario node in summer/fall 2017. Spleen, lung and liver samples from each deer were tested by reverse transcription (RT)-PCR. Tissues from PCR-positive animals were tested by serotype-specific conventional RT-PCR followed by Sanger sequencing for serotype identification.

**Results:** Sixteen carcasses were evaluated from September 7-November 28, 2017, including two of four white-tailed deer that died from August 29-September 7 at the same site in London, Ontario. Both had lesions consistent with orbiviral hemorrhagic disease, including multi-organ petechial and ecchymotic hemorrhages on serosal surfaces, most prominently in the walls of the pulmonary artery, abomasum and rumen. EHDV was isolated from spleen samples and spleen, lung and liver samples were positive for EHDV-2 by RT-PCR.

**Conclusions:** This report describes the first known occurrence of EHDV in east-central Canada. The timing of this outbreak overlapped with and may have been an extension of a larger EHDV outbreak across parts of the eastern U.S. Its detection in Ontario represented the northern-most recognized edge of the outbreak.

**D-112: CHARACTERIZATION OF ARTERIAL VASCULOPATHY IN JUVENILE NORTHERN ELEPHANT SEALS, ASSOCIATED WITH OTOSTRONGYLUS CIRCUMLITUS INFECTION**

Tzushan Yang, Tanja Zabka, Wes Baumgartner, Padraig Duignan, Cara Field

*Otostrongylus circumlitus* (Nematoda: Filarioidea, Metastrongyloidea) is the common lungworm of juvenile northern hemisphere phocid seals and a rare cause of mortality in endemic hosts. For the past two decades it has been associated with high morbidity and mortality in juvenile northern elephant seals (NES; *Mirounga angustirostris*). During the 2017 NES pupping season, otostrongyliasis accounted for 11% of NES admissions and 56% of the mortalities at The Marine Mammal Center. Death generally results from severe pulmonary hemorrhage and disseminated intravascular coagulation. However, the vascular pathology in NES has not been fully characterized. Lung sections from confirmed cases (n = 10) and uninfected controls (n = 3) were examined microscopically. The vasculopathy is characterized by moderate to severe concentric laminar medial hypertrophy and vacuolation in pulmonary artery or smaller vessels, variably associated with luminal stenosis and post stenotic capillary dilatation. Additional findings included acute to organizing thrombi; transmural and adventitial neutrophilic infiltrate with occasional karyorrhectic debris but without appreciable disruption or degeneration of the medial wall (periarteritis); and infrequently, endarteritis/subintimal inflammation and intraluminal nematodes. The vasculopathy has histologic features similar to that described in humans and domestic animals with pulmonary hypertension (PH). The pathogenesis of the vasculopathy may be associated with larval migration and chronic/recurrent thromboembolism. Thus, the PH is proposed to be a main contributing factor for the vasculopathy. Additional microscopic staining and prospective antemortem clinical assessment of PH of affected NES will be performed to further characterize the vasculopathy and to elucidate the proposed pathogenesis.

**D-113: SPIRORCHIDIASIS WITH CONCURRENT PYELOSOMUM COCHLEAR INFECTION IN A GREEN SEA TURTLE (CHELONIA MYDAS)**

Yen-Chi Chang, Yun-Chieh Tuan, Ji-Hang Yin, Hue-Ying Chiou

A juvenile Green sea turtle (*Chelonia mydas*) about 9.45 kg in body weight and 45 cm in body length, was presented to disease of wildlife monitoring group of National Chung Hsing University for post-mortem examination after found dead on the coast of Penghu, Taiwan. The turtle was in poor body condition. At necropsy, multifocal white to dark red nodules about 0.1 × 0.1 - 0.2 × 0.3 cm in size, were found on the serosal surface of multiple organs including cerebrum, heart, lungs, liver, spleen, kidney and gastrointestinal tract, with profuse dark-red effusion in the body cavity. An emboli with numerous digeneans about 0.7 × 0.1 cm in size, was found in artery of right forelimb. Digeneans about 0.3 × 0.2 × 0.1 in size with yellowish, tear-drop appearance were found on the endothelial surface of urine bladder. Microscopically, multifocal

necrogranulomatous inflammation were found in all of organs that we examined. The foci of inflammation were comprised of moderate to large numbers of epithelioid macrophages which surround brown trematode eggs about 40-60 µm in diameter with three-micrometer-thick shell. Moderate lymphoplasmocytic inflammation was observed in the endothelium of the urine bladder. Parasitic identification including morphological identification and molecular biological examination showed that the trematode in the vessel and urine bladder were *Haplotrema postorchis* and *Pyelosomum cochlear*, respectively. Base on the gross findings, histopathological examination, morphological identification and molecular biological examination of the parasites, final diagnosis of spirorchidiasis with concurrent *Pyelosomum cochlear* infection in a green sea turtle (*Chelonia mydas*) was made.

#### **D-114: FATAL LEPTOSPIROSIS WITH MUCOCUTANEOUS ULCERATION IN A FREE-RANGING RACCOON (PROCYON LOTOR) IN BRITISH COLUMBIA, CANADA**

Heindrich Snyman, Bruce Burton, Helen Schwantje, Ken Sojonky, Breanne Glinnum, Stephen Raverty, Tomy Joseph

A distressed adult male raccoon was reported to a local wildlife rehabilitation center. The raccoon was reluctant to use its hind limbs due to a large, ~ 3.0 × 4.5 cm deep necrotic wound along the left lateral surface of the perineum. The mucocutaneous margins of the lips and cheeks, hard and soft palate, nasal philtrum and nose pad, inner eyelids, and prepuce and penis all contained extensive, ~ 2.0 to 6.5 mm, coalescing shallow cavitated ulcers. Given a poor prognosis the animal was euthanized. Microscopically there was necro-suppurative interstitial nephritis and extensive adrenal necrosis which along with the perineal wound and mucocutaneous ulcers was filled with dense colonies of silver stained spirochetal bacteria. Leptospirosis was confirmed by immunohistochemistry and PCR. Virus isolation and PCR testing for Canine Distemper Virus, Feline Calicivirus, and consensus Parapox- and Herpes-virus was negative and there was no evidence of an underlying viral infection. *Leptospira* spp. is a zoonotic spirochetal bacterium that causes severe systemic disease in a variety of domestic animals. In most wildlife species Leptospirosis is typically limited to inapparent subclinical infections and a carrier state rather than clinical disease. Raccoons are especially common carriers and specifically serve as a maintenance host for *Leptospira interrogans* serovar grippityphosa. Clinical Leptospirosis in a raccoon in the absence of any predisposing immunosuppressive disease is exceedingly rare. Although mucous membranes represent a common site for transmission the severe ulceration observed in this raccoon represents a particularly unique presentation that has not been previously reported in any species.

## **Education Focused Scientific Session**

November 6, 2018 | 8:00 AM – 12:00 PM

Session Chair: Rachel Mo Peters, DACVP, Agios Pharmaceuticals, Arlington, MA and Mee-Ja Sula, DACVP, University of Tennessee, Knoxville, TN

November 6, 2018

11:15 AM – 11:30 AM

### **AN ACTIVE INTEGRATION EXERCISE FOR DEVELOPMENT OF ILLNESS SCRIPTS IN THE TEACHING OF ANATOMIC PATHOLOGY**

Gary Haldorson, Steven Hines

For years at Washington State University, we have been using a 3-way-match style format for exam questions in the Systemic Pathology course for second year veterinary students. In this question type, students are typically given a clinical scenario including signalment and brief history, then asked to match that scenario with 1) the name of a disease, 2) an anatomic lesion, and 3) a “mechanism” consisting of either a pathogenesis of the lesion or a pathophysiology of how the lesion causes clinical disease. The students are given a list of potential answers for the disease, lesion and mechanism, including a few distractor answers for each from which to choose.

As a means of demonstrating our expectations on the exams, and as an opportunity for students to practice this exam question type in small groups, we have been using a “gamified” exercise in our laboratories. In the form of a sorting game, the exercise provides the practice as well as offering feedback in a social, low-risk environment. The goal of this exercise is to assess pathology knowledge in the context of clinical disease, while reducing the testing of the “technique” of answering the exam questions, and instead lead the students to critically considering the actual answers.

In this presentation, I hope to briefly describe the exercise first, and provide an opportunity for the audience to actively engage in the actual exercise, as our students would do in lab.

November 6, 2018

11:30 AM – 11:45 AM

### **ERROR MANAGEMENT TRAINING AND METACOGNITION: INVESTIGATING THEIR EFFECT AND ROLE IN VETERINARY STUDENTS LEARNING BLOOD SMEAR ANALYSIS**

Danielle Meritet, Elena Gorman, Katy Townsend, Patrick Chappell, Duncan Russell

Training students commonly associate errors with negative outcomes. Error management training (EMT) endeavors to modify student perception of errors by encouraging mistakes, and reinforcing their critical role in effective learning. Our group previously demonstrated the value of EMT in students learning surgical knot tying and herein, we aim to determine the efficacy of EMT in canine blood smear preparation and interpretation. EMT was supplemented by a training module intended to promote metacognition. We hypothesized that EMT and metacognition are associated with improved performance in unfamiliar scenarios (adaptive transfer), as compared to error-

avoidant training (EAT). A total of 26 students were prospectively enrolled into this double blinded study. Performance was evaluated according to monolayer area, slide quality, cell identification, white blood cell differential count, and overall interpretation. Students were trained with a combination of static images and known, normal canine blood samples. All participants tested 72hrs after training demonstrated improved monolayer areas, slide quality and cell identification (paired t-test and Wilcoxon signed-rank test, all  $P < 0.001$ ). When encountered with an unknown blood sample, both EMT and EAT groups had no significant differences with regards to their differential counts and slide interpretation (t-test and Wilcoxon rank sum; two tailed  $P > 0.22$ ). Students who self-identified as having greater metacognition were associated with less accurate differential counts and interpretations at 6 weeks post-training but this difference was not statistically significant ( $P > 0.16$ ). These data suggest that EMT and strategies that emphasize metacognition can be used to effectively teach blood smear analysis.

November 6, 2018

11:45 AM – 12:00 PM

### **VIRTUAL REALITY IN THE CLINICAL PATHOLOGY CLASSROOM**

Melinda Camus, James Moore, Sun Ahn, Joshua Skelton, Megan Caudill, Bridgette Wells, Robert Forbes

Veterinary students are extremely adept at memorizing facts, with their primary aim being to recite them on multiple-choice examinations. To help them break these habits, a team of clinical pathologists, medical illustrators, software developers, adult education specialists, and a voice actor have created a virtual reality experience. In this modality, individual students are immersed in a virtual environment, starting in a laboratory setting in which they are able to examine blood smears on a virtual microscope. Then they are teleported into the canine bloodstream, where they capture circulating blood cells, examine them to learn about their functions, and compare their distribution to a virtual CBC. Once this orientation experience is completed, the dog develops an inflammatory nidus and the leukocytes begin to react accordingly. The students *time travel* to see first-hand the changes in the leukocyte populations and correlate these with the values in subsequent CBCs throughout the patient's treatment course. Because the timing and magnitude of the changes that occur in leukocyte populations differ between dogs and horses, students repeat the immersive process in a horse as it experiences an inflammatory insult. Veterinary students have beta tested these experiences and the efficacy of the virtual reality approach is being compared with presentation of the material using the traditional format of lecture and microscope-based sessions in the senior pathology rotation. Student perceptions and motivation also are being assessed through direct behavioral observation and self-reporting instruments.

## **Education Focused Scientific Poster Session**

### **ED-01: LOCAL PEER OBSERVATION OF TEACHING**

Diana Hassel, Elena Gorman, William Vernau, Brian Murphy, Suzanne Kurtz, Christiane Löhner

The Regional Teaching Academy (RTA) of the Consortium of West Region Colleges of Veterinary Medicine was founded in 2011. A working group (WG) within the RTA formed to develop instruments for peer observation of teaching at the member veterinary colleges. To this end, the WG has developed instruments for local peer observation of teaching both for large group, didactic lecture instruction, small group discussions, problem-based learning, and clinical teaching formats. The peer observation is designed as a three-step process: the in-class observation is preceded and followed by self-reflection and coaching of the instructor on specific goals for the session and outcomes, respectively. The instruments, user guide, and best practices for the peer observation process will be presented and are available to the public on the RTA website: <https://teachingacademy.westregioncvm.org/initiative-localpeerobservation/>. The instruments are in use at Oregon State University (OSU), Colorado State University (CSU) and the University of California, Davis. Peer observation (rather than evaluation) is currently recognized as part of the Promotion and Tenure process at CSU, OSU, Washington State University (WSU) and UC Davis. Feedback from users about the ease of use, utility and value of the instruments is overwhelmingly positive and is guiding further improvements of the instruments.

### **ED-02: TEACHING VETERINARY STUDENTS HOW TO CAPTURE DIGITAL IMAGES USING THEIR SMART PHONES IN A CYTOLOGY ELECTIVE COURSE**

Jessica Hokamp, Maxey Wellman

Digital cytology is becoming more widely available in some diagnostic laboratory settings. Teaching students how to capture high quality images using a smart phone might improve their ability to submit diagnostic images to a digital cytology service once they are in a practice setting. Second and third year veterinary students enrolled in a cytology elective class were asked to watch a YouTube video on how to capture an image using a light microscope and a smart phone prior to the first class. During their first class, students were then asked to capture a diagnostic image from a fine needle aspirate from a dog with peripheral lymphoma. Minimal instruction was given other than to select an area with intact cells and capture the image using the 1000X oil immersion objective, making sure there was sharp focus and optimal lighting. During the remaining sessions, students were taught what features contribute to a high quality diagnostic image, after which they were given the same slide as they received during the first class and asked to capture a high quality image. A grading rubric was created by two faculty members teaching in the course to evaluate the students' images taken during the first class, compared to later in the course. Almost all students were able to capture a diagnostic image by the end of the course, and image quality improved from the initial image to the final image for the majority of the students.

### **ED-03: IS BRINE FINE? EFFICACY OF A SATURATED SALT SOLUTION AS AN ALTERNATIVE TO FORMALIN FOR STORAGE OF ANATOMY SPECIMENS**

Jane Manfredi, Ioana Sonea, Dalen Agnew

**Background:** Formalin is a known carcinogen that has been a staple for maintaining specimens for teaching veterinary anatomy and pathology (A&P) for years. Students are increasingly reluctant to use formalin fixed specimens, impacting student competency in A&P. Saturated salt solutions have been recently used for specimen fixation and storage, but have never been compared to other solutions.

**Objectives:** To evaluate four storage solutions (formalin, saturated salt, Wardsafe, and Klotz solution) for A&P specimens, assessing students' preferences, formalin levels, and microbial growth.

**Methods:** Four previously formalin-fixed equine distal limbs were stored in separate tubs of each storage solution. Specimens were removed from the solutions, with veterinary students ranking their preferred specimens based on smell. A second experiment similarly evaluated fresh frozen equine distal limbs stored in saline for 2 months and used in laboratories during the first two weeks. Microbial growth and formalin levels were documented for all.

**Results:** No microbial growth was detected in the first experiment, but the fresh frozen limbs grew numerous *Marinilactibacillus* species. Students preferred specimens stored in Wardsafe versus formalin, but had no preference of Wardsafe over salt or Klotz. Nose and eye irritation were noted with formalin and Klotz. Formalin levels were over the acceptable limit in the formalin stored limb. Frozen specimens were acceptable to students.

**Conclusions:** All storage solutions prevented microbial growth in the formalin-fixed specimens. Wardsafe or brine should be used for previously fixed specimens. Short term use of salt to store non-fixed specimens is acceptable.

### **ED-04: CREATING 3-DIMENSIONAL MODELS FROM SERIAL HISTOLOGIC SECTIONS USING OPEN-SOURCE SOFTWARE**

T. William O'Neill, Christiane Löhner

**Background:** The use of 3-dimensional (3D) reconstructions is widespread in medicine as a way to obtain volumetric measurements and to create teaching models. Reconstructions in 3D are commonly created from radiologic scans and are limited by technical specifications of the imaging machine, properties of contrast agents used, the ability of human operators or computer algorithms to distinguish structures, and the distance between image slices. Histologic slides provide much greater detail and better morphology, however, pose additional problems due to inherent artifacts in processing and the lack of 3D metadata.

**Objective:** We tested the utility of an open-source software package to generate 3D reconstructions from H&E stained histologic slides.

**Methods:** Tumor-bearing lungs of mice from a toxicology study examining the disease outcomes of *in utero* exposure to dibenzo[def,p]chrysene in offspring were serial sectioned at 200 micrometer intervals. Slides were digitized using a slide scanner with a scanning area of 24mm by 36mm and a resolution of 3600 DPI. Micrographs were aligned in ImageJ and converted to 8-bit grayscale TIFF stacks. Stacks were imported into 3D Slicer and volumetric metadata calculated from the scanning area and distance between serial sections.

**Results:** Using a combination of computer-calculated thresholding and human input on each section, we generated 3D models that can be manipulated in real-time, digitally cross-sectioned, and provide quantifiable, volumetric measurements.

**Conclusion:** While the process is labor intensive, the generation of 3D models from histological slides may have application in teaching and research.

#### **ED-05: INVESTIGATING THE EFFECTS OF ERROR MANAGEMENT TRAINING ON VETERINARY STUDENTS LEARNING SURGICAL KNOT TYING**

Danielle Meritet, Katy Townsend, Elena Gorman, Patrick Chappell, Duncan Russell

While errors can be a powerful impetus for learning, conventional pedagogy often emphasizes error-avoidant strategies that reward 'correct' answers and disregard 'mistakes'. Error management training (EMT) takes an explicitly positive approach to errors, using them to create an active and self-directed learning environment. Using a surgical knot tying model, we aimed to determine the efficacy of EMT in veterinary students with no prior surgical experience. We hypothesized that EMT results in improved performance in unfamiliar scenarios (adaptive transfer), as compared to an error-avoidant method. In this prospective blinded study, forty two students were equally divided into error avoidance training (EAT) and EMT. Performance in instrument- and hand-tied knots was evaluated for technique, time, number of attempts and, where applicable, knot bursting strength. All participants demonstrated significant improvement between a pre-test and an analogous test 72hrs after training by all outcomes (paired T-test and Wilcoxon matched-pairs; two-tailed  $P$  all  $< 0.045$ ). An adaptive transfer test found no significant differences in outcomes between EMT and EAT at 72hrs ( $P$  all  $> 0.093$ ). All participants experienced a significant decline in 6/8 outcomes at 11 weeks post-training ( $P < 0.045$ ). This reduction in performance was driven by EAT in 5/6 outcomes. These data demonstrate that students trained in error management experience comparable gains in short term performance, including their ability in adaptive transfer. This may be retained after a sustained period of quiescence, perhaps at a better rate than students trained with error-avoidance. Educators may wish to more actively incorporate EMT into veterinary curricula.

#### **ED-06: EDUCATIONAL INSIGHTS FROM AN INTERCULTURAL PATHOLOGY STUDY ABROAD PROGRAM FOR VETERINARY STUDENTS**

L. Tiffany Lyle, Nozomi Shimonohara

An intercultural pathology study abroad program to Japan was initiated at Purdue University in summer 2018 with two primary goals: 1) provide students with a skillset to

collaborate and engage with veterinary professionals worldwide, and 2) engage students in veterinary pathology beyond the classroom. Two veterinary students and one pathology resident participated in the Intercultural Pathology Study Abroad Program. The program lasted for two weeks and was facilitated by two board certified veterinary pathologists, one from Purdue University and the other from IDEXX Laboratories in Japan. Students were exposed to many aspects of veterinary pathology ranging from zoo and wildlife pathology at the Ueno zoo, lab animal pathology within universities, and emerging infectious diseases at the National Institute of Infectious Diseases. During the study abroad experience students from Purdue University interacted daily with students from four universities in the metro Tokyo area. Purdue University students and students from Universities in Japan participated in group lab activities that included immunohistochemistry, mammalian necropsy, and mystery slide seminar cases. Analysis of intercultural learning and cultural self-awareness was based on day-to-day debriefings and reflective essays at various time-points throughout the program. All participants demonstrated an increased level of cultural self-awareness at the conclusion of the program. This program served as a phenomenal tool to engage veterinary students in global veterinary medicine through pathology. A global engagement skillset in the veterinary curriculum not only enhances learning, but is a boundless tool to encourage global collaboration and engagement early in the careers of veterinary students.

## **Veterinary Student Poster Session**

### **SP-01: PATHOLOGICAL LESIONS OF THE CAPTIVE BUDGETT'S FROG (LEPIDOBATRACHUS LAEVIS)**

Mandy Womble, Gregory Lewbart, Heather Shive

**Background:** *Lepidobatrachus laevis*, commonly called the Budgett's frog, is a member of the horned frog family (Ceratophryidae) that has become increasingly popular among amphibian hobbyists. Budgett's frogs are also used in developmental biological research, providing a novel model species for studying organogenesis, regeneration, evolution, and biological scaling. However, there is minimal scientific literature detailing disease processes or histologic lesions in this species.

**Objective:** Our objective was to describe spontaneous pathologic lesions in Budgett's frogs in order to identify disease phenotypes in this species.

**Methods:** We performed a retrospective analysis of 14 captive Budgett's frogs (wild-caught and captive-bred) autopsied at the NC State University College of Veterinary Medicine between 2008 and 2018.

**Results:** The majority of frogs exhibited renal lesions, including: varying combinations of tubular epithelial binucleation, karyomegaly, and cytoplasmic vacuolation; polycystic kidney disease; and renal carcinoma. A second common finding was variably sized, randomly distributed bile duct clusters (biliary proliferation). Other findings that were likely contributors to morbidity and mortality included regional or generalized edema; intestinal adenocarcinoma; aspiration pneumonia; and parasitism.

**Conclusion:** This retrospective analysis describes histologic lesions in captive Budgett's frogs, including identification of spontaneous neoplasia. Renal and liver lesions were the most frequent histologic changes. Interestingly, renal lesions resemble a condition in Japanese and Chinese toad hybrids that progresses from tubular epithelial atypia and tubular dilation, to polycystic kidney, to renal carcinoma. These findings provide novel insight into disease processes in captive Budgett's frog populations that may be helpful for clinical management and disease diagnosis in these animals.

### **SP-02: PLEOMORPHIC LIPOSARCOMA IN A DOG**

Stefanie Muller, Tanya LeRoith

Liposarcomas are uncommon soft tissue sarcomas of dogs and cats, which rarely metastasize, and most often arise from the subcutis. Surgical excision is the treatment of choice, and local recurrence is possible following incomplete resection. Histologically, four subtypes have been characterized: atypical lipoma, well-differentiated liposarcoma, myxoid liposarcoma, and pleomorphic liposarcoma. While in humans these subtypes have different biological activity, this has not been conclusively determined in small animals, although some studies suggest that pleomorphic liposarcomas are the most likely to metastasize. From 2013-2018, the Virginia Tech Animal Laboratory Services received 22 biopsies diagnosed as liposarcomas. The ages ranged from 6 to 13 years

old, with both an average and median age of 9.5 years old. Of these, two were further classified as pleomorphic liposarcomas. We present here a case of pleomorphic liposarcoma in a six-year-old, neutered male, mixed breed dog. Key features of pleomorphic liposarcomas displayed in this case included foamy, eosinophilic cytoplasm, intracytoplasmic lipid vacuoles (approximately 50% of cells in this case), marked variation in nuclear size and shape (round to oval to elongate), prominent nucleoli, and binucleate and multinucleate cells. Pleomorphic liposarcomas lack the mucin background of myxoid liposarcomas, and display more pleomorphism than well-differentiated liposarcomas (sheets of round to polygonal cells with variously sized lipid vacuoles) and atypical lipomas (increased cellularity of well-differentiated lipoblasts, and increased numbers of lipoblasts.)

#### **SP-04: CLONING CANINE CD16 FOR DIRECTED NK CELL-IMMUNOTHERAPY**

Chiara Fattori, Erin Dickerson, Antonella Borgatti, Kelly Makielski, Ashley Graef, Jamie Van Etten, Haeree Park, Jaime Modiano

Thanks to the similarities between various human and dog tumors, the dog could provide a model to study novel immunotherapy strategies.

NKs (Natural Killer Cells) are powerful cytotoxic effectors with great immunotherapeutic potential. In human medicine it is well known that CD16 is the most important NKs' activator and the only means of NK cells interaction with anti-neoplastic antibodies, in order to trigger Antibody-Dependent Cell-mediated Cytotoxicity (ADCC). However, the role of CD16 in dog NKs is unclear. The aims of this project were a) to confirm and quantify the expression of CD16 in dogs' white blood cells (WBCs), and b) to clone canine CD16 into a mammalian vector for subsequent transfection of mammalian cell lines.

WBCs from healthy dogs were used for mRNA extraction and cDNA retrotranscription. A) qRT-PCR was used to detect and to quantify CD16 expression. For cloning, primers were designed for the entire length of the gene and the PCR- technique was used to amplify the product of interest. By means of restriction enzymes, the gene was cut and cloned into the vector for expression in mammalian cells. The results confirmed the expression of CD16 in canine WBCs, and a strategy to clone CD16 was successful in generating a product. Cloning the product will allow for experiments to display canine CD16 to test its function and to generate specific reagents for its detection or manipulation, such as antibodies for enrichment of CD16+ cells and scFV (single-chain variable fragment) to engage CD16+ cells using multi-specific antibodies or killer entities.

#### **SP-05: MOST FREQUENT INJURIES SUSTAINED BY FREE-RANGE CATS KILLED IN ROAD TRAFFIC ACCIDENTS IN ST. KITTS**

Samantha Zayas, Judit Wulcan, Michelle Dennis

Worldwide, road transportation is a life-threatening risk for free-range cats. In developing countries, as St Kitts, where road conditions and cultural attitudes towards

road use and animals differ, research characterizing injuries of cats involved in road traffic accidents (RTA) is limited. RTA-associated trauma is common among feline patients presenting for emergency veterinary care in St. Kitts; therefore, a cross-sectional study was performed to determine the prevalence of injuries in cats killed by RTA. Commuters were asked to call-in any dead cats seen on the roadside from January 2018 to August 2018. An investigator scanned and collected the cats and documented their geographical location. Microchipped and/or severely autolyzed animals were excluded. A necropsy was performed on each cat with gross lesions documented and photographed. Demographics (n=20) were evenly distributed between male and female cats, all intact and most less than one-year-old. Bone fractures were uncommon and mostly restricted to the mandible, maxilla, and pelvis. Visceral displacement included diaphragmatic herniation, abdominal eventration, and external abdominal hernia. The most common visceral injuries were multiple liver lacerations, single splenic lacerations, locally extensive pulmonary contusions, and traumatic proptosis. Subcutaneous hematomas were seen, however, usually associated with dermal abrasions or bone fractures. This study is intended to be a benchmark for veterinarians during their physical examination and initial diagnostic evaluation of the feline patient presenting with vehicular injuries. With the prevalence of RTA-associated injuries in free-range cats on St Kitts described, further studies can evaluate the medical approach towards such patients for a better prognosis.

#### **SP-06: SALIVARY GLAND NEOPLASMS IN NON-HUMAN PRIMATES: A CASE SERIES AND BRIEF LITERATURE REVIEW**

Emily Howard, Olga Gonzalez, Sanjeev Gumber, Daniel Anderson, Shyamesh Kumar, Edward Dick Jr

**Background:** Salivary gland neoplasms are rarely reported in non-human primates.

**Methods:** Pathology records for salivary gland neoplasms were reviewed for all necropsies over a 35 year period at Southwest National Primate Research Center and Yerkes National Primate Research Center. An in-depth literature search for all published cases of salivary gland neoplasms in non-human primates was performed.

**Results:** Seventeen salivary gland neoplasms were identified in eight rhesus macaques, six baboons, a chimpanzee, a Bonnet macaque, and a tamarin. Seven animals were female and six were male; sex was not available in four animals. Seven neoplasms were malignant (five adenocarcinomas, one carcinoma and one carcinosarcoma). Four had metastatic lesions in the lymph nodes or lung; another was locally invasive. Nine were benign (eight adenomas and a mucoepidermoid cystadenoma). One neoplasm, a mixed tumor, was of undetermined malignancy. Malignant neoplasms were seen more often in males. The most frequent origin was the parotid (n = 7), mandibular (n = 4) or minor (n = 2) salivary glands; four were of undetermined origin. Two animals with salivary adenoma had a history of prior radiation exposure.

**Conclusions:** Salivary neoplasms are relatively uncommon in non-human primates. The parotid salivary gland appears most frequently affected, with adenomas and

adenocarcinomas occurring most often. Salivary neoplasms should be considered in the differential diagnosis of head and neck masses, particularly in animals with a history of prior radiation exposure.

#### **SP-07: HAPTOGLOBIN AS AN INDICATOR OF ON-GOING ACUTE INFLAMMATION IN PRE-SLAUGHTER COWS**

Kabirat Okeowo, Funmilola Thomas, Eyitayo Ajibola, Olukayode Akintunde, Samson Rahman

**Background:** Haptoglobin (Hp) is a major acute phase protein (APP) of cattle and its measurement has been exploited in the diagnosis and prognosis of various diseases in bovines.

**Objective:** The goal of this study was to apply the evaluation of Hp levels to determine the presence of on-going inflammation in animals meant for human consumption.

**Methods:** Seventy three serum samples obtained from immediately pre-slaughtered cows in a major slaughter slab located in the South Western Nigerian city, Abeokuta, were analysed using ELISA for Hp. Haematological parameters were also analysed and a physical examination assessment of each animal's body condition was carried out.

**Results:** Hp was detected in moderate levels in 56% of animals examined. The range of values for Hp concentration was from 31.58 to 117.40ng/ml with a median of 45.05ng/ml. There were no significant correlations between the Hp concentrations and the haematology profile or the scores for physical examination assessment of the body condition of the cattle.

**Conclusions:** Overall, it is suggestive that the increases in Hp seen in blood of these animals may be more related to stress induced on the animals from transportation and conditions in the pre-slaughter holding pens, although the emergence of new acute inflammatory episodes cannot be totally ruled out. Assessment of other stress markers and a broader panel of acute inflammation indices would further shed light and add value to benefits of Hp profile as an indicator of acute inflammation in pre-slaughter animals.

#### **SP-08: OPTIMIZATION OF METHODS TO STUDY THE EFFECT OF CANNABINOIDS ON IMMUNE RESPONSES IN STIMULATED CANINE PBMCs**

Clare Brown, Evangel Kummari, Todd Archer, Barbara Kaplan

Current canine autoimmune disease treatments are not ideal due to limited efficacy or adverse side effects. Cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), are compounds from the marijuana plant (*Cannabis spp.*) that have gained attention as potential treatments for autoimmune diseases. In mice it has been established that they are immunosuppressive and provide benefit in autoimmune models, but in most other species there exists little data. The aim of this project was to examine the potential of cannabinoids as effective treatments for canine autoimmune diseases by determining whether they are immunosuppressive in canine peripheral blood mononuclear cells (PBMCs) in vitro. PBMCs were isolated from canine

blood and treated with CBD or THC, followed by stimulation with ConA, LPS, or PMA/ionomycin (PI). Carboxyfluorescein succinimidyl ester (CFSE) staining was used in conjunction with flow cytometry to assess lymphocyte proliferation. RNA was also isolated to determine gene expression of cytokines interleukin-2 (IL-2) and interferon gamma (IFN-gamma) via qPCR. After assessing PBMC responsiveness under various conditions, the studies focused on PI as it was the most effective stimulant. Results from both flow cytometry and qPCR indicated that CBD and THC could affect proliferation and cytokine production, but that it is dog-specific; some dogs exhibited no effect, while others exhibited modest suppression by CBD and THC. IFN-gamma consistently exhibited a higher magnitude of expression change than IL-2. These findings provide important preliminary insight into the potential of these compounds for canine autoimmune diseases and establish a knowledge base of effective methods for future studies.

#### **SP-09: ANTIBODY MEDIATED PROTECTION AGAINST RSV AFTER IMMUNIZATION WITH A VSV-G AND F RECOMBINANT**

Chaunte Lewis, Kelsey Brakel, Olivia Harder, Stefan Niewiesk

Respiratory Syncytial Virus (RSV) is an enveloped ssRNA virus that causes severe upper respiratory tract disease in young, immunocompromised, and geriatric patients. While this disease has a high mortality rate in these demographics, there is no licensed RSV vaccine available. Previous studies have shown that the fusion (F) glycoprotein is a potential vaccine target as it is necessary for infectivity. This study aims to explore whether or not a combination of two glycoproteins would confer protection, and whether or not this protection was B cell or T cell mediated. The fusion glycoprotein and the attachment glycoprotein (G) were utilized because they are both envelope proteins, and are the target for neutralizing antibodies and T cells. A recombinant vesicular stomatitis virus was generated which expressed both the F and the G protein, and tested in cotton rats which display increased susceptibility to RSV compared to mice. Cotton rats were inoculated with the recombinant VSV twice, and then challenged with RSV. Immunization with the VSV recombinant lead to the induction of antibody and a reduction in viral titer of 1.5 log. Even groups in which CD4+ T cells or CD8+ T cells, or both CD4+ and CD8+ T cells were depleted by antibody treatment were protected against infection with RSV. These findings indicated that the RSV-specific antibodies were the main correlate of protection against challenge with RSV. In summary, our study found that VSV-G and F recombinants are protective and able to induce an antibody response.

#### **SP-10: A SELECTIVE BCL-2 INHIBITOR IMPAIRS CANINE LYMPHOMA CELL LINE SURVIVAL**

Gillian Herbert, Tess Lawhon, Robert Gogal, Kristina Meichner

Canine Lymphoma (cL) is a leading cause of cancer-related death in dogs. Current gold standard treatment is a multi-agent chemotherapy protocol centered around doxorubicin, a potent single agent drug against cL. Despite initial favorable responses to treatment, the majority of dogs with cL succumb to their disease indicating the need for alternative treatment strategies. BCL-2 is an anti-apoptotic protein and critical for cell

survival. Venetoclax (ABT-199), an oral selective BCL-2 inhibitor, was recently approved for use in humans with chronic lymphocytic leukemia. This study evaluated the effects of venetoclax on cell growth, viability, apoptosis and cell cycle in a canine B-cell lymphoma (17-71) cell line. Cells were cultured for 72h in the presence of venetoclax or doxorubicin at increasing concentrations to establish half-maximal inhibitory concentrations (IC50). Cells grown with IC20 and IC50 concentrations were stained with 7-aminoactinomycin D (7-AAD) and annexin V to measure early and late apoptotic and necrotic cells, or with propidium iodide (PI) for cell cycle analysis and then analyzed by flow cytometry. Venetoclax inhibited cell proliferation, while cell enumeration analysis indicated venetoclax IC50 was more cytotoxic than doxorubicin. Further, a numerically higher proportion of venetoclax-treated cells were in the early and late stages of apoptosis compared to either untreated or doxorubicin-treated cells. Venetoclax disrupted the G0/G1, S and G2/M phases of the cell cycle. Overall, these preliminary *in vitro* findings suggest that venetoclax represents a promising treatment approach to improve outcome in dogs with lymphoma. Investigations in four other canine lymphoma cell lines are currently ongoing.

#### **SP-11: THE EFFECT OF IRRADIATED HUMAN SQUAMOUS CELL CARCINOMA CELLS ON MICROGLIA ACTIVATION AND SURVIVAL**

Rachel Martin, Jessica Lawrence, Ali Khammanivong, Linh Nguyen, Gwen Phung, Davis Seelig

Previous studies in mouse models have demonstrated neuroinflammation following radiation therapy (RT) targeting distant (i.e. non-CNS) tissue. The mechanism leading to this inflammation is unknown. The hypothesis is that inflammatory mediators produced by irradiated tissue lead to neuroinflammation and contribute to the cognitive deficits seen in cancer patients prescribed non-brain RT. The purpose of this study was to identify and measure cytokines produced by human squamous cell carcinoma cells (TR146) after irradiation and determine their effect on human microglia (HMC3). Growth curves and radiation dose-response were determined to define the ideal culture media and target radiation doses. To simulate the effect of peripheral tumor treatment on microglia, media from irradiated TR146 cells (0, 3, 6, 9, and 12 Gy) was transferred to microglia at 1, 6, and 24 hours (h) post-treatment. To determine microglial viability, MTS assay was performed 48h following serum transfer. To determine changes in their activation state, microglia were stained for Iba1 expression 48h after serum transfer. TR146 cells had decreased dose-related viability and clonogenic survival after radiation. Viability of microglial cells following media transfer from irradiated TR146 cells did not demonstrate a clear dose-related decrease in viability. Future work will include measurement of cytokine production from media collected over time from irradiated TR146 cells and co-culture of microglial cells with irradiated TR146. Results will inform the underlying mechanisms contributing to non-brain radiation related cognitive dysfunction and permit strategic intervention to mitigate radiation side effects.

#### **SP-12: NOVEL IN SITU HYBRIDIZATION ASSAY FOR THE DIAGNOSIS OF CHAGAS MYOCARDITIS AND ORCHITIS IN A RHESUS MACAQUE**

Megan DeLorenzo, Elvira Carias, Allison Mustonen, Luis Giavedoni, Olga Gonzalez, Shyamesh Kumar, Edward Dick Jr

**Background:** A five-year-old, male, rhesus macaque was presented with a history of lethargy, dehydration, and scrotal edema. Animal was euthanized, and necropsy was performed. Histopathology identified lymphoplasmacytic myocarditis and orchitis with intralesional *T. cruzi* cysts and amastigotes.

**Methods:** Formalin fixed paraffin embedded serial sections were stained with H&E, and RNAscope In Situ Hybridization (ISH) assay was performed using a probe specific for the kinetoplast minicircle repeat DNA sequences.

**Results:** RNAscope based ISH showed intense positive signals in the heart and testicle. In the heart, ISH labeled the intracellular cysts and isolated foci within the areas of inflammation with no visible cysts. Additionally, ISH labeled several cardiac myocyte nuclei and perinuclear cytoplasm. In the testes, ISH labeled the intraepithelial cysts as well as individualized amastigotes in the lumen of the seminiferous tubules.

**Conclusions:** We present the first case of *T. cruzi* induced orchitis in a rhesus macaque. Additionally, we present a novel diagnostic method for the detection of cysts and amastigotes in fixed tissues. Finding *T. cruzi* in the testis supports the potential for sexual transmission.

### **SP-13: TH2 CELLS AND ILC2 RESPONSES IN ALLERGIC WEST HIGHLAND WHITE TERRIERS**

Valeria Bergomi, Elia Tait-Wojno, Simon Frueh

Canine allergy is characterized by inappropriate inflammation in response to harmless environmental substances. Diagnosis and treatment are challenging, and the immunological mechanisms that underpin disease remain unclear. Thus, investigating immune pathways of allergy may inform the development of novel diagnosis and treatment modalities. Human and murine studies have shown that immune cells called CD4<sup>+</sup> Th2 cells and Innate Lymphoid Cells 2 (ILC2s) play important roles in allergic responses. Th2 and ILC2 cells secrete type 2 cytokines in response to allergens; while Th2s are antigen specific, ILC2s lack antigen specificity. The respective contribution of ILC2s and Th2 cells to the allergic immune response in dogs is unknown. In this study, we quantified Th2 cells and ILC2s in the blood of allergic and healthy West Highland White Terriers, which are predisposed to allergy. Demographic, diagnostic, and clinical information was collected for each participating dog. PBMCs from each dog were subjected to flow cytometric analysis, employing Gata3 to identify Th2 cells and ILC2s. The frequencies and total numbers of Th2 cells and ILC2s were compared between groups. Allergic dogs had a higher percentage of Th2 cells and a lower percentage of ILC2s. Neither difference was statistically significant with the small sample size used in this initial study, and analysis of a larger number of samples is ongoing. This study highlights the importance of Th2 cells as major immunological players in canine allergic disease, and our data set the stage for future investigation in how these cells could be targeted to treat disease.

#### **SP-14: IMPAIRED EXPRESSION OF AP ENDONUCLEASE-1 IMPAIRS FETAL DEVELOPMENT AND LEADS TO EMBRYONIC LOSS**

Andrew Oates, Gerco den Hartog, Lindsay Butcher, Amber Ablack, Mason Matsubara, Tadahide Izumi, Sheila Crowe, Peter Ernst

**Background:** Apurinic/apyrimidinic endonuclease-1 (APE-1) is induced by the accumulation of reactive oxygen species (ROS) and plays multiple roles in response to oxidative stress including: regulation of redox-sensitive genes; inhibition of ROS accumulation; inhibition of apoptosis and; repair of abasic sites in oxidative DNA damage. While a complete APE-1 knockout (KO) is embryonic lethal, a novel hypomorphic (HM) mouse model was developed in which APE-1 expression was inhibited ~80%. The goal of this project was to test the hypothesis that reduced expression of APE-1 in HM embryos causes fetal loss during organogenesis.

**Methods:** MRI was used as an in vivo model to assess embryonic viability, and necropsies were performed at specific gestational time points. PCR was used to determine genotype, and western blot was used to assess APE-1 expression. Histology was performed, and samples were stained with H&E and immunofluorescence.

**Results:** Breeding of heterozygous (Het) mice yielded ~50% of the HM offspring compared to what would be predicted by Mendelian genetics. Mating HM males with Het females showed a higher failure:viable fetus ratio than Het-Het pairings (~3:5 vs ~1:8). HM mice were smaller than littermates at all gestation periods measured, and APE-1 expression was reduced. Gross evaluation of embryonic/fetal failure suggests multiple mechanisms are at play. Analysis of histology is ongoing.

**Future Directions:** Additional samples and assays will be required to provide the power to support definitive conclusions. Histology and immunofluorescence have been performed, and will be assessed for tissue specific APE-1 expression and pathology.

#### **SP-15: A MORPHOLOGIC STUDY OF 17 CASES OF PERIOCLAR XANTHOGRANULOMAS IN DOGS**

Alexandra Harvey, Leandro Teixeira, Richard Dubielzig

**Purpose:** To evaluate the clinical, histopathologic, and immunohistochemical features of 17 cases of periocular xanthogranulomas diagnosed in dogs from archived cases from the Comparative Ocular Pathology Laboratory of Wisconsin (COPLOW).

**Methods:** Archived records from COPLOW were searched for cases of canine periocular xanthogranulomas. Cases were evaluated for the presence of lipid-laden macrophages and Touton giant cells. Seventeen cases matching those criteria were identified (1993–2018).

**Results:** Clinically, periocular xanthogranulomas in dogs presented as small bland nodules. They commonly occurred at the limbus (8/17) or cornea (4/17). Three of 17 cases were less than 3-years-old. Nine of 17 cases occurred in males, while 5/17 were in females and 2/17 were unspecified. Fourteen of 17 cases did not report any lipid or

metabolic abnormalities. Histologically, the lesions were composed of dense populations of macrophages with abundant vacuolated cytoplasm and Touton giant cells with minimal background inflammation and an intact overlying epithelium. All cases demonstrated positive immunohistochemical labeling for CD204. Cholesterol clefts were seen in only two cases.

**Conclusions:** Xanthogranuloma has been used to describe a variety of histiocytic lesions characterized by abundant lipid-laden macrophages. The authors use the term, periocular xanthogranuloma, to describe nodules with rigidly defined cellular characteristics. These distinct characteristics set them apart from previously described solid intraocular xanthogranulomas. Although these lesions share many characteristics with human limbal xanthogranulomas, further investigation is needed before there is enough data to suggest different subsets as reported in the medical literature; in particular, a careful characterization of the metabolic health of affected animals.

#### **SP-16: EFFECTS OF LIVER CELL SURFACE GLYCOCONJUGATES ON INVASION BY PLASMODIUM BERGHEI**

Aumbriel Schwirian, Jonas King

The initial interaction between malaria sporozoites and host liver cell has become the focus for a novel malaria vaccination. The current recombinant vaccine (RTS,S/AS01) targets the point of hepatocyte invasion, but fails to block parasite transmission reliably. Parasite invasion is still poorly understood. Interaction between the sporozoite circumsporozoite protein and host hepatocyte heparin sulfate proteoglycans (HSPGs) is believed to facilitate invasion; however, other currently unknown redundant pathways involving glycoconjugates targeted by sporozoites for invasion are suspected. To investigate these pathways, HepG2 human hepatic carcinoma cells were grown in the presence of swainsonine, a Golgi alpha-mannosidase inhibitor, or *HS2ST1* siRNA, a silencer of the heparin sulfate 2-O-sulfotransferase 1 gene. These were chosen to evaluate how glycoconjugates interact with sporozoites. Determining a dose that would yield reduced HSPG / N-glycan levels while maintaining high cell viability was a critical first step. Cells were dosed with a range of 0.02 µg/mL - 10 µg/mL of swainsonine to determine changes in cellular morphology and cell viability. No changes were detected with fluorescent microscopy at these concentrations. Cells were also incubated with 100 µg/mL swainsonine or the siRNA and stained with fluorescent lectins to evaluate changes in cell surface glycoconjugates. Global effects of silencing *HS2ST1* are to be evaluated with real time qPCR. While this project is still in its beginning stages, it is expected that altering the cell surface glycoconjugates will have a negative effect on *Plasmodium* invasion.

#### **SP-17: A RETROSPECTIVE ANALYSIS OF POSTMORTEM EQUINE RENAL LESIONS**

Caitlin Culligan, Linden Craig, Jennifer Janes

Significant renal disease is uncommon in equine patients, comprising up to only 1% of hospitalized horses. There are very little data on the epidemiology of acute versus chronic renal failure and the characterization of renal disease in horses. This

retrospective study aims to determine the most common renal lesions at postmortem examination, the distribution of primary versus secondary lesions, and the most frequent causes of identified renal lesions in horses submitted for autopsy. A search of the pathology records from the University of Kentucky's Veterinary Diagnostic Lab identified 387 out of 10,541 equine autopsies and biopsies from January 2010 to June 2018 with renal disease (3.7% of total cases). Of these cases, 49.6% were female and 50.4% were male. Of the cases examined, renal lesions were determined to be primary in 35% of cases and secondary to another process in 65% of cases. Nephritis was the most common lesion type in both primary and secondary groups, comprising 56% and 86.5% of cases, respectively. Tubulointerstitial nephritis was the most common nephritis type (36.2%), followed by interstitial nephritis (35.3%), tubulonephritis (14.7%), embolic nephritis (5.5%), glomerulonephritis (4.6%), and glomerulointerstitial nephritis (3.7%). Leptospirosis was the most common primary cause of death (47%) with all cases in fetal or juvenile horses. *Leptospira pomona* was the most frequently identified serovar. Other causes of renal lesions included renal infarctions (8.5%), tubular proteinosis (7.2%), hydronephrosis (6.46%), nephrolithiasis (4.1%), renal neoplasms (2.8%), renal cysts (2.8%), nephrocalcinosis (2.8%), *Klossiella equi* infection (1.6%), and polycystic kidney disease (1.3%).

#### **SP-18: LATS1 AND LATS2 MAINTAIN THE FATE OF ADRENOCORTICAL CELLS IN THE EMBRYONIC ADRENAL GLAND**

Amelie Menard, Adrien Levasseur, Guillaume St-Jean, Marie Le Gad-Le Roy, Marie-Odile Benoit-Biancamano, Derek Boerboom, Alexandre Boyer

In the Hippo signaling pathway, the Large tumor suppressor kinases 1 and 2 (LATS1/2) are functionally redundant serine/threonine-protein kinases that phosphorylate and inhibit YAP and TAZ, which are transcriptional coactivators that play a major role in the regulation of cell proliferation and differentiation during embryonic development. In order to investigate the role of Hippo signaling in adrenal gland development, we generated a mouse model (*Lats1<sup>flox/flox</sup>;Lats2<sup>flox/flox</sup>;Nr5a1<sup>cre/+</sup>*) in which *Lats1* and *Lats2* were conditionally deleted during adrenocortical development. *Lats1<sup>flox/flox</sup>;Lats2<sup>flox/flox</sup>;Nr5a1<sup>cre/+</sup>* mice were characterized by a progressive accumulation of spindle-shaped cells in the adrenal cortex, leading to adrenal failure and death by 2 to 3 weeks of age. Immunohistochemistry performed on adrenal glands from embryonic day 14.5 (E14.5), E17.5 and 1 day post partum (1dpp) animals showed a progressive appearance of cells expressing SMA, suggesting that the spindle-shaped cells are myofibroblasts. An increase in the expression levels of *Acta2*, *Cald1*, *Cnn1*, *Cox2* and *Spp1* was also observed, confirming the myofibroblastic nature of the spindle-shaped cells. Furthermore, a marked nuclear accumulation of YAP and TAZ in the myofibroblasts, the absence of an increase in apoptotic cells and a decrease in the mRNA levels of steroidogenic genes were observed in the adrenal glands of *Lats1<sup>flox/flox</sup>;Lats2<sup>flox/flox</sup>;Nr5a1<sup>cre/+</sup>* mice. A similar phenotype was also observed in the testes. These results suggest that LATS1 and LATS2 normally act to restrict YAP and TAZ activity, thereby maintaining proper steroidogenic cell fate and inhibiting their transdifferentiation into myofibroblasts. These results define a crucial role for Hippo signaling in adrenocortical and testis development.

## **SP-19: CLINICAL, HISTOPATHOLOGICAL, AND IMMUNOHISTOCHEMICAL FEATURES OF CANINE SPLENIC STROMAL SARCOMAS**

Katherine Murphy, Elizabeth Anglin, Jennifer Mahoney, Amy Durham, Molly Church

**Background:** Primary splenic stromal sarcomas are common in dogs. This heterogeneous group of sarcomas exclude lymphoma, histiocytic sarcoma, and hemangiosarcoma, but are not further well-defined. Prior reviews of this spectrum of sarcomas include outdated nomenclature and cursory immunohistochemical staining panels. Reports of clinical behavior are limited, though few have indicated a high metastatic incidence and identified mitotic index as a means to determine behavior.

**Objective:** This retrospective study's aims are to characterize the histopathological and immunohistochemical features for better classification of splenic stromal sarcomas, and correlate with clinical outcomes.

**Methods:** Fifty-three cases of splenic stromal sarcoma were evaluated. Clinical data from medical records included therapy following splenectomy, survival information, and disease progression. Histopathologic slides were evaluated for morphologic features, necrosis, metastatic spread, and mitotic index. For study inclusion, the immunohistochemical panel included IBA1, CD204, smooth muscle actin, and CD31 to rule-out histiocytic sarcoma and hemangiosarcoma.

**Results:** Patient survival information was available for 48/53 cases. The overall median survival time (MST) was 213 days. The MST for patients with splenectomy only was 162 days, and 293 days for dogs with surgery and chemotherapy. Metastasis to the liver was confirmed in 25% (12/48) of cases. Mean and median mitotic index was similar between tumors with and without confirmed metastatic disease (mean=13.8 and 13.9, respectively; median=5.5 and 7.0, respectively). Degree of necrosis was not predictive of metastatic spread.

**Conclusions:** We found improved clinical outcomes, namely MST and metastatic incidence, with more refined characterization of splenic stromal sarcomas in dogs.

## **SP-20: HEMOPHAGOCYTIC HISTIOCYTIC SARCOMA IN A DOG**

Mitchell Meyerhoeffer, Brooke Robertson, Kurt Zimmerman, Sheryl Coutermarsh-Ott, Thomas Cecere

A 10 year-old, male castrated, Golden Retriever presenting to the Virginia-Maryland College of Veterinary Medicine Veterinary Teaching Hospital with a two week history of regenerative anemia, lethargy, and hyporexia. Abdominal ultrasound revealed enlargement of the liver, an enlarged spleen with multifocal splenic nodules, and enlarged hepatic and splenic lymph nodes. Aspirates of all three organs revealed a hemophagocytic syndrome presumed to be a neoplastic process. The patient was subsequently euthanized and submitted for necropsy. Necropsy revealed a diffusely and markedly enlarged liver, a diffusely enlarged and meaty spleen with multifocal raised nodules, and enlarged hepatic and splenic lymph nodes. Histologically the liver, spleen, and hepatic/splenic lymph nodes contained a neoplastic population of round to

polygonal cells, which frequently contained cytoplasmic hemosiderin or erythrocytes (erythrophagia). Neoplastic cells diffusely exhibited cytoplasmic immunoreactivity against Iba1. These findings support a diagnosis of hemophagocytic histiocytic sarcoma (HS). Although the majority of histiocytic sarcomas are of interstitial dendritic cell origin, hemophagocytic HS is unique among histiocytic diseases in that it arises from CD11d positive macrophages that are resident in the spleen and bone marrow.

## **SP-21: PHOTOGRAPHY COMPARISON BETWEEN DSLR AND SMARTPHONE CAMERAS IN ANATOMIC PATHOLOGY**

Haley Blazek, Bradley Njaa

High quality, gross images are critical for pathology instruction and publishing. Numerous digital platforms are available and the options continue to expand with variation in type of device, device convenience, image quality, and input costs. With the vast majority of people using smartphones, a side-by-side comparison between professional quality DSLR cameras and smartphone digital cameras was needed and became the purpose of this study. The DSLR camera used was a Nikon D7200 with a Tamron SP AF 60mm F/2 and a Hoya rotating polarizing filter. The smartphones used were a Google Pixel I with a single 12.3 megapixel rear camera and an iPhone 7 Plus with dual 12-megapixel rear cameras. Both were used with a clip-on SANDMARC rotating polarizing filter. For consistency, all photos were taken on a glass top photo stand with a black background and two, daylight balanced, Hensel C-light D metal-vapor lamps with rotatable polarizing gels. Tissues were selected with a variety of lesions, textures, focal distances, and a wide range of colors. Images were assessed for quality, overall focus, depth of field, and background. Photographs of larger specimens were as good, and in some cases better, with the smartphones, especially when using portrait mode on the iPhone 7 Plus. Smaller specimens tended to have more glare or reflection from the background using the smartphone cameras. Dark red specimens were variable and inconsistent between the DSLR and smartphone cameras. In certain instances, smartphone images had equal or better quality when compared to the DSLR images.

## **SP-22: PERIPHERAL BLOOD ABNORMALITIES IN A CAT RECEIVING CANINE XENOTRANSFUSION**

Natasha Taylor, Eric Fish, Pete Christopherson, Emily Graff, Laura Lowe, Lenore Bacek

A stray 3-year-old, spayed, female domestic shorthair cat presented to the AUVTH emergency service after being rescued from a parking lot. On physical examination, the patient was obtunded, hypothermic and icteric, hyperlactatemic (15.7 mmol/L), and severely anemic (PCV 14%). FIV/FeLV testing were negative. Multiple IV crystalloid boluses were given to stabilize the patient. Additional diagnostics were not initially performed for financial reasons. Due to economic constraints, a 50 mL canine packed red blood cell transfusion was administered to treat the anemia. A complete blood count performed 48 hours later revealed improved PCV (29.8%), slight reticulocytosis (59,400/uL), marked leukocytosis (48,610/uL) and platelets within reference interval (313,000/uL). Two distinct groups of RBCs were identified on the Advia 120 V/HC scatterplot. A pathology review of the blood smear identified many large canine erythrocytes, moderate numbers of smaller normal feline erythrocytes, predominantly

feline leukocytes, and evidence of microangiopathy (schistocytes and acanthocytes). Serum biochemistry analysis revealed moderate to marked hyperbilirubinemia (6.43 mg/dL), increased ALT, AST, and ALP, and a markedly increased hemolysis index. Doxycycline, dexamethasone SP, fenbendazole, and nitenpyram were initiated for empiric treatment of hemoparasites and/or Immune Mediated Hemolytic Anemia (IMHA). The cat was stable for two days before experiencing a sudden drop in PCV from 30% to 14%, and the patient became neurologically abnormal. Peracute hemorrhage and/or thromboembolism was suspected. The patient was humanely euthanized; a necropsy was not performed. This case describes the unique hematologic findings and sequelae to xenotransfusion in veterinary medicine.

### **SP-23: BENIGN VS MALIGNANT TRICHOEPITHELIOMAS: A RETROSPECTIVE ANALYSIS OF 73 CASES (2014-2018)**

Scott Mitchell, Sheryl Coutermarsh-Ott

Trichoepitheliomas are adnexal tumors that are quite common in the dog and are most often benign. Less commonly they will exhibit malignant behavior characterized by local invasion and/or metastasis. We performed a retrospective analysis and identified 73 excisional biopsies from 64 canine patients diagnosed as trichoepitheliomas. Sixty-one of these were diagnosed as benign and thirteen were diagnosed as malignant. Nine patients had more than one trichoepithelioma diagnosed, all of which were benign. The most common breed reported was the Golden Retriever, with 17.7 percent of benign cases and 23.0 percent of malignant cases being associated with that breed. Females accounted for 68.6 percent of patients diagnosed with the benign form, and 77.0 percent of those diagnosed with the malignant form. The mean age of patients at diagnosis was 7.94 years for benign tumors, and 8.58 years for malignant tumors. Both benign and malignant tumors occurred most commonly on the mid to lower limbs (20.6 percent of cases) and shoulders (16.4 percent of cases). The mean area of the masses at excision was shown to be 27.4 cm cubed for the benign form, and 118.8 cm cubed for the malignant form. Histologically, benign tumors tended to be partially or fully encapsulated, while malignant tumors tended to be unencapsulated. The average mitotic index for the benign tumors was lower than that of the malignant tumors. In review of previous literature on these neoplasms, our demographic findings are consistent with other studies, however no histologic data comparing benign and malignant tumors was found.

### **SP-24: BLOOD-BRAIN BARRIER PATHOLOGY IN AN EXPERIMENTAL MODEL OF BLAST-INDUCED NEUROTRAUMA**

Christa Cheatham, My Hoang, Gozde Uzunalli, Seth Herr, Riya Shi, L. Lyle

The signature injury of modern warfare is blast-induced neurotrauma (BINT) following improvise explosive device (IED) exposure. The clinical symptoms of BINT have been correlated with neuropathology including edema, hemorrhage, neuronal necrosis, and increased paracellular permeability of the blood-brain barrier (BBB). Herein, we aim to investigate the pathological alterations of the BBB in rats following single or repeated blast exposure. The BBB is composed of endothelial cells with tight junctions, a basement membrane, pericytes, and astrocyte endfeet. BINT was induced by using an

open-ended blast apparatus to deliver single or triple 150 kPA shockwaves to 3-month-old male Sprague Dawley rats. After 24 hours, the brains were excised, flash frozen, and cryosectioned into 5µm thick sections. Qualitative and quantitative evaluation of the BBB was accomplished using immunofluorescence microscopy and Zen blue analysis software. We observed alterations in expression with endothelial cells, tight junction proteins, tight junction adaptor proteins, basement membrane, and pericytes. Our preliminary findings demonstrated a 1.82-fold ( $p=0.037$ ) increase in the expression of claudin-5, a tight junction protein, in the single blast model compared to the control. There was a 2.01-fold ( $p=0.018$ ) increase in the expression of PDGFR- $\beta$ , a pan-pericyte protein, in the single blast model compared to the control. The triple blast model demonstrated a 1.87-fold decrease ( $p=0.028$ ) in PDGFR- $\beta$  expression compared to that of the single blast. This is the first comprehensive pathologic analysis of the BBB in BINT in an experimental model. These data will support the development of a robust and reproducible experimental model of blast-induced neurotrauma.

**SP-25: MOLECULAR, MORPHOLOGIC, AND ULTRASTRUCTURAL CHARACTERIZATION OF INTESTINAL NEMATODES IN PANAMANIAN GOLDEN FROGS (ATELOPUS ZETEKI) FROM THE MARYLAND ZOO IN BALTIMORE**

Julia Purnell, Sarah Poynton, Lisa Mangus

The Maryland Zoo in Baltimore houses the largest captive assurance colony of critically-endangered Panamanian Golden Frogs (PGFs) in North America. PGFs from this population frequently carry low to moderate numbers of intestinal nematodes that were hypothesized to belong to the family Cosmocercidae based on histologic features. In this study, we used molecular, morphologic, and ultrastructural methods to test this hypothesis and achieve more specific classification of the worms. Using degenerate primers targeting the nematode 18S rRNA gene, we performed polymerase chain reaction and sequencing on DNA isolated from fecal samples and paraffin-embedded tissues. Resulting sequences were aligned with nematode sequences in GenBank using the BLAST tool and consistently returned top matches with *Raillietnema*, a genus in the family Cosmocercidae known to infect amphibians and reptiles. Light microscopy of whole worms revealed key features of *Raillietnema*: transverse striations of the cuticle, tapered tail, rhabditiform esophagus, and relatively few (up to 19) large ( $61.68 \pm 5.44$  micrometers  $\times$   $41.14 \pm 4.23$  micrometers) larvated ova in the uterus. Scanning electron microscopy showed three large lips, cuticular striations at a periodicity of 1-2 micrometers, and mamillated somatic papillae 3 micrometers in diameter. To the authors' knowledge, this is the first detailed description of nematodes infecting PGFs. Although the nematodes are not typically associated with significant pathologic changes in PGFs from the Maryland Zoo, accurate identification of the worms is critical for future efforts to release captive-bred PGFs into their native habitat, as introduction of non-native parasites could have deleterious effects on other endemic wildlife.

**SP-26: IN SITU ILEAL EXPRESSION OF IL-10 AND IFNGAMMA DURING EARLY INTESTINAL MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION OF CALVES**

Latasha Ludwig, Rebecca Egan, Kevin Stinson, Brandon Plattner

Innate immunity plays an important role in the outcome of mycobacterial infections in many species. Oral exposure to *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) in cattle is widespread, but only a minority of exposed individuals develop clinical disease. The intestinal environment is important in early host defense; however, the specific mechanisms remain unknown. Gamma delta T cells are common at mucosal surfaces including the small intestine, and produce a wide range of cytokines including IFN $\gamma$  (IFN $\gamma$ ) and IL-10. IFN $\gamma$  is pro-inflammatory and promotes macrophage activation, while IL-10 is immunoregulatory and inhibits inflammation and promotes its resolution. Our objective in this study was to characterize the production of these two cytokines *in situ* during early localized intestinal *Map* infection with the goal of understanding the local immune mechanisms contributing to early pathogen clearance in the intestine. We used RNAscope technology and spectral microscopy to detect IFN $\gamma$  and IL-10 transcripts in the ileum after experimental *Map* infection. IL-10 was always more abundant than IFN $\gamma$  in all sections of *Map*-exposed and unexposed calves. IL-10 expression was more abundant in Peyer's patches compared with the lamina propria, and at most time points we detected more IL-10 transcripts in *Map*-exposed calves compared to unexposed controls. We also co-labelled CD3 cells in a subset of calves to show that the majority of IL-10 was produced by CD3(neg) cells. Further characterization of *in situ* cytokine and immune cell characterization should be conducted to better understand the mechanisms in early clearance of mycobacterial infections.

## **SP-27: CLINICAL AND HISTOPATHOLOGIC FEATURES OF EXFOLIATIVE CUTANEOUS LUPUS ERYTHEMATOSUS IN FOUR GERMAN SHORTHAIRED POINTERS**

Patrick Huang, Verena Affolter

Exfoliative cutaneous lupus erythematosus (ECLE) of German shorthaired pointer (GSP) and Vizsla is a rare form of Lupus erythematosus. This retrospective study reviews the clinical and histologic features of ECLE in four GSP referred to the Veterinary Medical Teaching Hospital at University of California, Davis. The age upon presentation ranged from seven months to four years and 3/4 dogs were female. All dogs had a history of skin lesions largely unresponsive to antibiotics and corticosteroids. Exam findings included multifocal to diffuse areas of alopecia and scaling, with variable crusting, erosions, ulcerations, leukoderma, pruritus, and erythema. The dorsum was affected in all four cases. Additional lesions were seen on the ventrum, axillae, inguinal areas, limbs, muzzle, and nasal planum. Consistent histologic findings included lamellar and parakeratotic hyperkeratosis and lympho-plasmacytic interface/cytotoxic dermatitis, characterized by vacuolar change of basement membrane zone and basal cells, dispersed necrotic/apoptotic keratinocytes and scattered melanin-laden macrophages. Lesions extended into the follicular epithelia (mural folliculitis) and sebaceous glands were absent. Treatment with immunosuppressive agents and antibiotics resulted in improvement of clinical signs in 1/4 dogs. Disease progressed in 1/4 dogs due to owner discontinuing medications and 1/4 dogs was euthanized within two years due to declining quality of life. The fourth dog passed away from unknown causes; no necropsy was performed. A genome-wide association study has identified a single nucleotide polymorphism in the canine chromosome 18 associated with familial ECLE in GSP's.

Further studies are needed to identify the mutant gene responsible for this disease process.

#### **SP-28: CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF FELINE DIFFUSE IRIS MELANOMA**

Maylin Akella, Ian Herring, Andrew Enders, Kayla Waler, Tanya LeRoith

This report describes the clinical presentations and the histopathological findings of feline diffuse iris melanoma (DIM). Of twenty-two feline globes submitted to Virginia Tech Animal Laboratory Services (ViTALS) at Virginia-Maryland College of Veterinary Medicine since 2013, seven were diagnosed histologically as feline DIM. In all cases, the owners elected enucleation due to progressive iris pigmentation, regions of raised iridal pigmentation and/or secondary elevated intraocular pressure. Histologic findings ranged from benign iridal hyperpigmentation to iris melanoma with scleral and choroidal invasion. Feline DIM is a progressive disease with a highly variable rate of progression, and it is difficult to predict the metastatic potential of the neoplasm. Clinically, extended pigmentation to the drainage angle, exfoliated cells in the aqueous humor, glaucoma, and involvement of posterior iris epithelium are commonly used as the indicators of higher risk of metastasis. Histopathologically, high mitotic index, tumor necrosis, extrascleral extension, and choroidal invasion are associated with increased metastatic rate. Here, we compare the clinical presentations and histopathological characteristics in feline DIM.

#### **SP-29: INVESTIGATION OF BUBR1 EXPRESSION IN ZEBRAFISH CANCERS**

Jesse Riker, Heather Shive

The tumor suppressor genes *TP53* and *BRCA2* are essential for maintenance of genomic integrity, and mutations in these genes result in genomic instability. One consequence of genomic instability is aneuploidy (aberrant number of chromosomes). Genomic stability is dependent in part on the spindle assembly checkpoint (SAC), which ensures accurate chromosomal segregation and is mediated by a complex of proteins that includes the kinase BUBR1. Interestingly, mutations in *TP53* and *BRCA2* each influence the level of BUBR1 expression in cancers from humans and mice: while mutant *TP53* is associated with increased levels of BUBR1, mutant *BRCA2* is associated with decreased levels of BUBR1. Levels of BUBR1 expression in cancers have also been linked to diploid versus aneuploid status. To further explore the relationship between *BRCA2* and *TP53* mutations, BUBR1 expression, and ploidy in cancers, we are characterizing *bubr1* expression in zebrafish cancers via immunohistochemistry, Western Blot, and RT-PCR. We will examine cancers from *tp53*-mutant zebrafish (*tp53 m/m*) with or without concurrent *brca2* mutation (*brca2 m/m;tp53 m/m*) and compare these findings to ploidy status of cancers. It is expected that cancers will exhibit variable levels of *bubr1* expressions that are correlated to both genotype (*tp53 m/m* or *brca2 m/m;tp53 m/m*) and ploidy outcome (diploid or aneuploid). Overall this study will provide further insight on the interplay of conserved molecular contributors to carcinogenesis.

### **SP-30: PLATELET-ENDOTHELIAL ASSOCIATIONS MAY IMPACT THE PATHOGENESIS OF CYTOMEGALOVIRUS INFECTION IN MICE**

Brenna Daly, Alicia Braxton, Griffin Cyphers, Shefali Vijay, Jacqueline Brockhurst, Alyssa Chalmin, Kevin Najarro, Karl Johnson, Catherine Cryer, Selena Guerrero-Martin, Rupkatha Mukhopadhyay, Yu-Pin Su, Ravit Boger, Kelly Metcalf Pate

Platelet decline is a hallmark of many acute viral infections, including cytomegalovirus (CMV) infection in humans and mice. Specific mechanisms of platelet decline, such as platelet sequestration in interactions with other cells, can directly impact viral pathogenesis through receptor- and cytokine-mediated signaling. The immunopathogenesis of platelet decline in CMV infection is incompletely understood. Preliminary data show that experimental platelet depletion in CMV-infected mice prevents CMV replication in salivary glands that occurs in non-depleted mice 21 days post-infection. It has also been demonstrated that platelet sequestration in monocyte, lymphocyte, or neutrophil aggregates does not cause platelet decline in CMV infection. We hypothesized that platelet-endothelial associations (PEAs) contribute to platelet decline during acute CMV infection in mice and that PEAs, if present, form when circulating platelets are required for CMV replication in salivary glands. Forty-two juvenile male BALB/c mice infected with  $3 \times 10^6$  plaque-forming units of CMV were euthanized on days 0, 3, 8, or 21 for tissue collection. To detect PEAs in salivary glands, immunohistochemistry was used to label platelets with anti-CD41 antibody and a hematoxylin counterstain was used to visualize tissue architecture. The percentage of PEA-positive vessels increased following CMV infection and was statistically significant 21 days post-infection compared to uninfected controls ( $P=0.0125$ ). This suggests that PEA formation contributes to platelet decline and occurs concurrently with viral replication in salivary glands. Future work will assess whether PEAs are required for CMV replication and determine if inhibiting PEA formation could decrease CMV replication in infected patients.

### **SP-31: EVALUATION OF CYTOKINE PRODUCTION BY HUMAN NATURAL KILLER CELLS EXPRESSING ENGINEERED FC RECEPTORS**

Ally Rogich, Kristin Snyder, Robert Hullsiek, Daniel Mendez, Hemant Mishra, Jimmy Wu, Bruce Walcheck

There has been great progress in developing cancer immunotherapies, but resistance in patients and the non-responsiveness of some malignancies present challenges. This is the case for ovarian cancer in which antibody-based and cell-based immunotherapies have not been successful for this solid tumor. Natural Killer (NK) cells, innate lymphoid cells, are efficient at recognizing antibodies attached to tumor cells and killing these cells by a process known as Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC). Human NK cells exclusively recognize tumor-bound antibodies by their receptor FcγRIIIA (CD16A). CD16A, however, is a low affinity receptor, which may limit the anti-tumor activity of NK cells. The FcγR CD64 binds to antibodies with much higher affinity than CD16A, but CD64 is not expressed by NK cells. We generated a novel chimeric FcγR composed of the extracellular region of CD64 and the intracellular region of CD16A, referred to here as CD64/16A. We hypothesized that NK cells expressing CD64/16A would produce higher levels of cytokines than NK cells expressing CD16A

upon engaging antibody-bound ovarian cancer cells. An important anti-tumor cytokine produced by NK cells is interferon gamma (IFN $\gamma$ ). Cytokine production was evaluated following ADCC and collection of NK cell supernatants. Extracellular cytokines were quantified by the use of a Cytometric Bead Array ELISA and flow cytometry. The CD64/16A NK cells produce more IFN $\gamma$  in the presence of mAb. Future implications of my studies are the use of NK cells expressing CD64/16A as a based immunotherapy for ovarian cancer and other malignancies in combination with therapeutic antibodies that induce ADCC.

### **SP-32: ACUTE LEUKEMIA IN A BEARDED DRAGON (POGONA VITTICEPS)**

Jessica Wild, Carolina Azevedo, Randi Gold, Richard Ploeg, Tracy Stokol, Rachel Cianciolo, Edward Calomeni, Brooke Griff, Sharman Hoppes, Mary Nabity

An adult intact female bearded dragon lizard was presented to the Zoological Medicine service for anorexia. A complete blood count revealed an extreme leukocytosis with predominantly intermediate to large mononuclear cells, consistent with an acute leukemia. Due to the poor prognosis, the patient was euthanized and a necropsy was performed. Gross necropsy findings showed a large diffusely pale tan friable liver with no obvious masses. Histopathologic evaluation demonstrated diffuse infiltration of large mononuclear round cells in the bone marrow, heart, lung, liver, kidney, spleen, and brain. A diagnosis of acute leukemia was made. Additional diagnostic tests were performed to identify cell lineage, including immunohistochemical (CD3) and cytochemical staining and transmission electron microscopy (TEM). Tumor cells were negative for CD3 and 47% had multifocal granular chloracetate esterase staining (CAE). Monocytes from a control bearded dragon were diffusely positive for CAE. Cells lacked features of myeloid cells on TEM. A presumptive diagnosis of acute lymphoid leukemia was made. Although lymphoma is the most common type of cancer in lizards, acute leukemia is rarely reported. This case demonstrates the use of various methods in an attempt to classify the type of leukemia.

### **SP-33: COMPARISON OF DETECTION METHODS FOR EQUINE HERPESVIRUS-1 PREVALENCE IN HEALTHY ONTARIO BROODMARES**

Michaela Botts, Carina Cooper, Jutta Hammermueller, Luis Arroyo, Brandon Lillie

Equine herpesvirus (EHV) 1 is ubiquitous in equine populations worldwide and can cause late gestation abortion, neonatal death, respiratory disease, and myeloencephalopathy. There is both a modified live and killed vaccine commercially available that have been proven to decrease the duration of viremia, level of viral shedding, and clinical signs, however, repeated outbreaks still occur in vaccinated herds. Over 350 healthy broodmares were sampled bimonthly to evaluate EHV shedding and humoral response status throughout a 12-month cycle. Samples collected included: nasal and vaginal swabs to gauge viral shedding, whole blood to identify viremia and latent infections, and serum for antibody levels. In previous prevalence studies, different methods of assessment were used resulting in poor repeatability, and no assessment of vaginal swabs has been conducted. Our overall project investigated the hypothesis that current vaccination protocols do not decrease the risk of viral shedding in healthy broodmares, and that infected broodmares will experience viremia,

and shed virus intermittently, while also comparing three different sampling sites(nasal, vaginal, blood). Results show that an average of 37.1% of clinically healthy mares in Ontario yield at least one positive sample at any given timepoint, and that positivity in one sample does not correlate well with positivity in other samples as 87.9% of mares that were positive at a given timepoint were only positive in one of the three samples. Varying results across different testing methods may explain why prevalence results are widely variable between studies, and that multiple tissue testing is better for tracking true prevalence.

#### **SP-34: THE RAPID GROWTH OF A BIOLOGICALLY AGGRESSIVE, BUT HISTOLOGICALLY BENIGN FIBROSARCOMA IN A DOG**

Jasmine Choi, Clinson Lui, Spring Halland, Ana Alcaraz

A five-year-old castrated male German shorthaired pointer cross presented with a small oral mass associated with the upper right canine tooth. The mass was initially unnoticeable on the outside of the mouth. Dental prophylaxis with several tooth extractions was performed and samples of the mass with associated bone were submitted for biopsy. Histopathology results suspected fibrosarcoma of the maxilla with periosteal new bone formation. The dog was started on non-steroidal anti-inflammatory drugs to slow the growth of the tumor. Five months after the initial findings, the patient had difficulty eating due to an expansive growth that distorted the right side of the face. The mass invaded the gum, causing loss of almost all of the teeth. Euthanasia was elected due to the severity of the condition. Necropsy findings revealed a 10x12cm mass that deformed the right maxillary area and bulged from the hard palate extending from the incisive dental pad to the soft palate. Based on the accelerated growth of the tumor and the gross and histopathologic findings, the mass was consistent with fibrosarcoma, described as biologically aggressive but histologically benign. Although uncommon, fibrosarcomas have been diagnosed in dogs, particularly in medium-to-large breed dogs.

#### **SP-35: JEJUNAL CARCINOSARCOMA IN A GOLDEN RETRIEVER**

Natalie Tocco, Peter Moak, Ann Mack, Shannon Martinson

Carcinosarcomas are rare tumors in dogs and most often involve the mammary, apocrine, salivary, or thyroid glands. An 8 year old Golden Retriever presented to a local clinic for gastrointestinal signs and a palpable abdominal mass. An 8-10 cm long mass involving the proximal jejunum was detected on exploratory laparotomy prompting jejunal resection and anastomosis. The mass was submitted to Diagnostic Services at the Atlantic Veterinary College for histopathology. Histologically the highly invasive tumor consisted of both an epithelial and a mesenchymal component. The epithelial cells were scant forming small islands and often exhibited signet ring cell morphology. Mucoid secretory material within the cytoplasm of these cells was PAS positive and the cells were positive for pancytokeratin and negative for vimentin and KIT (CD117) using immunohistochemistry. The mesenchymal population formed the bulk of the mass and consisted of spindle-shaped to rhomboidal cells that were often embedded in cartilaginous matrix. These cells were positive for vimentin using immunohistochemistry and negative for cytokeratin and KIT. Based on histopathology and

immunohistochemistry, the tumor was diagnosed as a jejunal carcinosarcoma. This is the first report of intestinal carcinosarcoma in a dog.

### **SP-36: SUBLINGUAL MASS IN A DOG: WHAT ELSE IF NOT A STORAGE DISEASE?**

Samantha Zayas, Nair Rajeev, Mary Anna Thrall, Pompei Bolfa, Anibal Armien

A two-year-old intact male mixed breed dog presented with sublingual masses, emaciation, and vomiting to the veterinary school clinic. An initial biopsy of the masses revealed diffuse severe pyogranulomatous glossitis (macrophages being often massively distended by foamy to clear cytoplasm), multifocal ulceration, with fibrin deposition, mild lymphoplasmacytic infiltration and lymphangiectasis. The dog's poor prognosis and therapeutic response resulted in its euthanasia. Autopsy revealed bilateral thyroid hyperplasia, mild hydrocephalus with white matter atrophy, aboral esophageal dilation, multifocal pyloric ulcers, mild nephrosis, and cachexia. Enlargement of lymph nodes, vagosympathetic trunk, cervical nerves, and dorsal root ganglia were also noted. Microscopically, neuronal cell bodies of the central nervous system were enlarged, vacuolated, and degenerated with accompanying perivascular storage, astrocytosis, and microgliosis. Enlarged, vacuolated neurons were also observed throughout the submucosal and mesenteric plexus of the gastrointestinal tract along with gut-associated lymphoid depletion and finely vacuolated macrophages infiltrating the gastric and intestinal lamina propria. Electron microscopy showed the cytoplasmic vacuolation to contain amorphous granular electron dense to optically vacant electron lucent material. The working diagnosis for this case was of lysosomal storage diseases other than mucopolysaccharidoses.

### **SP-37: SUBCONJUNCTIVAL HEMORRHAGE IN 147 DOGS**

Unity Jeffery, Jannina Saastamoinen

Animal abuse is a threat to animal welfare, and perpetrators frequently pose a risk to humans. To both identify abuse and avoid false allegations, veterinary pathologists must be aware of the patterns of injury associated with abuse and alternative explanations for these lesions. Scleral hemorrhage (also termed subconjunctival hemorrhage) has been identified as a potential sign of non-accidental injury in dogs, but there is little available information about differential diagnoses. The objective of this retrospective case series was to determine the causes of scleral hemorrhage in dogs presenting to an American veterinary teaching hospital. Electronic medical records of dogs presenting between 2007 and 2017 were searched and 147 cases of scleral hemorrhage identified. Of these, 119 had a history of trauma. The most common types of injury were vehicular trauma (47/119) and dog attacks (32/119). Confirmed or suspected non-accidental injury was reported for 5/119. Of the 28 dogs without a history of trauma, 13/28 had a bleeding disorder and of these, immune-mediated thrombocytopenia was the most common diagnosis (9/13). Other causes included seizure disorders (4/28), vasculitis (3/28) and ocular or periocular disease (3/28). This case series demonstrates that while scleral hemorrhage can be associated with non-accidental injuries, caution must be used before concluding that abuse has occurred.

because there are a variety of accidental injuries and underlying diseases that should be ruled out.

### **SP-38: INVESTIGATION OF ECHINOCOCCUS MULTILOCULARIS IN CALGARY MUSKRATS**

Marian Trudeau, Collin Lelain, Samantha Stamler, Alessandro Massolo, Jamie Rothenburger

The tapeworm, *Echinococcus multilocularis*, is of emerging importance for human and canine health in Alberta, Canada. The parasite life cycle involves predator-prey relationships between canids and rodents, while incidental ingestion of eggs can cause alveolar echinococcosis in humans and domestic dogs. Although some rodent intermediate hosts in local urban ecosystems are known, the role of muskrats (*Ondatra zibethicus*) in the ecology of *E. multilocularis* is unknown in North America. In Europe, invasive urban muskrats are considered important maintenance hosts. In 2017, 93 muskrats trapped in urban marshes in Calgary, AB were submitted for diagnostic investigation of liver abnormalities. The overall objectives of this study were to 1) confirm the presence of *E. multilocularis* using gross and histopathology, 2) quantify infection severity, and 3) assess demographic risk factors. Among this sample, 22.6% had lesions of alveolar echinococcosis; many were patent infections containing protoscoleces. Affected organs included liver, mesentery, spleen, reproductive organs, omentum and kidney. For liver cysts, the mean number was 2 (range 1-4) and the mean total surface area was 2513.91 mm<sup>2</sup> (range 28.27 mm<sup>2</sup> - 6964.13 mm<sup>2</sup>). No risk factors were significantly associated with infection with univariable logistic regression (sex, body condition, body mass;  $p > 0.05$ ). The size and invasive nature of these lesions likely has a negative impact on individual muskrat health. The high prevalence of fertile infections, concurrent with the known predation on muskrats by urban coyotes, indicate that muskrats may play a role in parasite transmission in urban settings, with important implications for disease control initiatives.

### **SP-39: FIRST REPORT OF WEISSELLA CETI AN EMERGING PATHOGEN CAUSING HIGH MORTALITIES IN RAINBOW TROUT ONCORHYNCHUS MYKISS IN COLOMBIA**

Gersson Vásquez, Jersson Avila, Miguel Rubiano

The present study reports the first outbreak of Weisellois by *Weissella ceti* in rainbow trout *Oncorhynchus mykiss* in Colombia between May and December 2017. The signs and lesions were mainly found in trout over 100g and consisted of lethargy, swimming on the surface or end of the ponds. Macroscopically, bilateral exophthalmia, periocular and intraocular hemorrhage, lenticular opacity, corneal rupture and blindness were observed. Petechial and ecchymotic hemorrhages were also observed on the basis of fins and skin. At necropsy, petechiae were randomly distributed in the liver and swim bladder and diffuse hemorrhages in the gastrointestinal tract including the anus.

The histopathological changes in the eye consisted of severe multifocal lymphocytic infiltration and scarce macrophages in choroids, retina and adipose tissue, accompanied by corneal ulceration, hemorrhages and cataracts. In some animals, a

large amount of coccobacilli in the foci of injury were seen. Moderate to severe multifocal mixed inflammatory infiltrate of lymphocyte predominance in skin, heart, liver, meninx, swim bladder, intestinal serous, pancreas rectum and anus.

The pathogen was isolated from eye, brain kidney and liver on TSA and BHI agar. *Weissella ceti* was confirmed by amplification and sequencing of the 16S gene of ribosomal RNA. Antibigram showed a high resistance to most antimicrobial agents. Only ampicillin susceptibility was found.

This is the first Weissellosis report on rainbow trout in Colombia. Currently our company is working on the development and implementation of a vaccine for the prevention of the disease.

#### **SP-40: CHARACTERIZATION OF BRONCHOPNEUMONIA WITH INTERSTITIAL PNEUMONIA IN FEEDLOT CATTLE**

Lauren Sergejewich, Laura Bassel, Dani Smerek, Kent Fenton, Jeff Caswell

Bronchopneumonia with interstitial pneumonia (BIP) is the third leading cause of mortality in beef feedlots. BIP is uniquely characterized by bronchopneumonia affecting the cranioventral lung and interstitial damage in the caudal lung. The latter lesion resembles acute interstitial pneumonia (AIP) although the epidemiological features differ. Little is currently known about the cause or nature of the disease. The aim of this study was to describe the pathological features of BIP and compare the cranial and caudal lung lesions to bronchopneumonia and AIP respectively. Lung samples collected from mortalities in feedlots were evaluated by histopathology, bacterial and mycoplasma culture, and virology. The cranial lung lesions in BIP cases were similar to those seen in cases of bronchopneumonia. Inflammatory cells and fibrin in airspaces as well as bronchiolitis obliterans were dominant features of the cranial lung sections. These cranial bronchopneumonia lesions were usually chronic and contained a variety of pathogens including *Mycoplasma bovis*. Oat cells and *Mannheimia haemolytica* genotype 2 were present in the cranial lung sections of fewer BIP cases compared to bronchopneumonia cases. Histologically, the caudal lung lesions were not significantly different from those in acute interstitial pneumonia, and included bronchiolar necrosis, alveolar hyaline membranes or type II pneumocyte proliferation, hemorrhage into airspaces and interstitial hypercellularity. Unlike the cranial lung, the caudal interstitial pneumonia lesions were usually acute. These lesions suggest a condition involving concurrence of chronic bronchopneumonia and acute interstitial pneumonia in the same animal.

#### **SP-41: METASTATIC OSTEOSARCOMA IN A VEILED CHAMELEON (CHAMAELEO CALYPTRATUS)**

Alexandra Ford, Emi Sasaki, Nobuko Wakamatsu

**Background:** Primary bone tumors such as osteosarcomas are rarely reported in reptiles. The behavior and likelihood of metastasis of osteosarcomas is well understood in other animal species, but not in reptiles, as it is not a common finding. Diagnosing

osteosarcoma histologically is through identification of spindle shaped neoplastic cells that produce an osteoid matrix.

**Case Description:** A 5-year-old male veiled chameleon in thin body condition was submitted for necropsy after a history of hypocalcemia and suspected nutritional secondary hyperparathyroidism. During necropsy an irregular, yellow soft nodule measuring 6 x 5 x 4 mm was observed attached to the coelomic wall over the 10<sup>th</sup> thoracic vertebrae and right 10<sup>th</sup> rib. Nodules of the same color and consistency but smaller in size were present on the coelomic wall along the caudal vertebrae. Histopathologic examination revealed the coelomic nodule to be an unencapsulated, infiltrative neoplasm of spindle cells with a homogenous, eosinophilic extracellular matrix, compatible with osteoid. Neoplastic cells exhibited moderate anisocytosis and anisokaryosis, 3 mitoses per 10 high-power fields, indistinct borders, and basophilic cytoplasm, and elongated nuclei containing 1-2 nucleoli. The heart, trachea, lung, liver, kidney, spleen, stomach, and adrenal gland were similarly affected. No bone involvement of the neoplasm was visualized.

**Summary:** This is a case report of a chameleon with a metastatic osteosarcoma, and the primary site remained undetermined. This type of tumor is rare in reptile species, and not well documented. Diagnosis was based upon the histological findings of neoplastic spindle cells with eosinophilic extracellular matrix compatible with osteoid.

#### **SP-42: SUBINVOLUTION OF PLACENTAL SITES IN A BITCH**

Jessica Magnotti, Vanessa Oakes, Phillip Sponenberg

A 5 year old, intact female Doberman pinscher dog presented to the Veterinary Teaching Hospital in lateral recumbency where her status deteriorated and she died spontaneously. She had delivered five puppies 7 weeks previously via cesarean section.

Gross examination of the uterus demonstrated zonal, dark red, raised foci within the endometrium. On histology, coalescing foci of acellular eosinophilic debris admixed with occasional degenerate neutrophils, rare foamy macrophages, and foci of hemorrhage expand and compress the endometrium. Trophoblasts are along the deep periphery, at the junction with the underlying endometrial surface.

These lesions are consistent with the clinical entity known as subinvolution of placental sites, or SIPS. This is an abnormal repair of endometrial placental sites resulting from either a failure or delay in normal uterine involution. This condition is seen most commonly in young bitches after their first litter. The underlying cause is currently unknown.

This condition is characterized clinically by the presence of hemorrhagic vaginal discharge persisting beyond 4 weeks postpartum, and histologically by the presence of invasive, trophoblast-like cells in the uterus up to 12 weeks after parturition. These cells commonly demonstrate deeper penetration than is typical of trophoblasts and have been occasionally reported to perforate through the uterine wall. This propensity for

deep endometrial penetration differentiates this diagnosis from the histologic appearance of normal placental involution.

#### **SP-43: ELUCIDATING THE MECHANISM AND DOSE REQUIREMENTS OF CPG ODN FOR THE PREVENTION OF OSTEOSARCOMA LUNG METASTASIS**

Robert Eisemann, Tara Piech, Timothy Fan, Kathryn Wycislo

**Background:** Osteosarcoma is the most common primary bone tumor in dogs and is estimated to impact over 10,000 canines each year. Unfortunately, the median survival time for dogs with osteosarcoma has plateaued and new therapies are desperately needed. Previous research by our group has identified the toll-like receptor agonist CpG ODN as an effective therapy for attenuating osteosarcoma lung metastasis and increasing survival time in a preclinical murine model of osteosarcoma.

**Objective:** We sought to determine CpG ODN's mechanism of action by investigating whether certain immune cells or pro-inflammatory cytokines are critical for the efficacy of CpG ODN. In addition, we aimed to define the minimal effective dose of CpG ODN to help guide dosage regimens in future canine clinical trials.

**Methods:** Using the K7M2 osteosarcoma cell line, in vivo experimental metastasis assays were performed with immunodeficient mice (NOD SCID and athymic nude) to determine if specific immune cell subsets are functional in CpG ODN's mechanism of action. TNF-alpha depletion and dose-response experiments were also employed using immunocompetent mice (BALB/c). Metastatic lung tumor burden was evaluated grossly and histologically.

**Results:** Immunodeficient mouse and TNF-alpha depletion experiments failed to reverse the metastatic attenuation observed with CpG ODN therapy. Immunocompetent mouse experiments identified a minimal effective dose of 25 micrograms CpG ODN/mouse.

**Conclusions:** While CpG ODN does appear to have a distinct minimal effective dose, its mechanism of action does not appear to be dependent on specific immune cell subsets or pro-inflammatory cytokines, such as TNF-alpha.

#### **SP-44: AN UNUSUAL PATTERN OF ENDOCARDIAL MINERALIZATION IN A YOUNG CAT WITH MITRAL VALVE DYSPLASIA**

Elise Hennessy, Jey Koehler, Jiwoong Her, Daniel Newhard, Darin Kepler

A one-year-old, intact male, Domestic Shorthair cat presented with a grade IV/VI parasternal systolic murmur, a one-day history of sudden onset respiratory distress, and a longer history of weight loss and poor hair coat. On thoracic radiographs, the cat had left-sided cardiomegaly and pulmonary edema. On echocardiogram, there was left ventricular dilation and concentric hypertrophy, with moderate thickening of the mitral valve leaflets, thickened chordae tendinae with abnormal connections to the papillary muscle, severe mitral regurgitation, and marked left atrial dilation. At necropsy, the

mitral valve and myocardial lesions seen on echocardiogram were observed, and the heart weighed 42 grams. In addition, there was a raised, plaque-like, irregular, rough, white-tan, firm, 1-cm diameter lesion on the endocardium of the interventricular septum immediately subjacent to the mitral valve. Histologically, this area had extensive deposition of brightly eosinophilic material with the same tinctorial quality as bone, in a linear, laminated pattern surrounded by fibrosis and mild granulomatous inflammation with multinucleated giant cells. This unusual and visually striking lesion likely represents dystrophic mineralization secondary to turbulent blood flow and regurgitation through a dysplastic mitral valve.

#### **SP-45: NASOPHARYNGEAL NEPHROBLASTOMA OF A 3 MONTH OLD GOAT**

Jillian Athey, L Rice, A Harvey, K Washburn, A Rodrigues Hoffmann

A three-month old Boer goat presented for respiratory issues, and thoracic radiographs showed interstitial pneumonia and mild bronchopneumonia. The goat was treated with albuterol and flunixin meglumine. The goat progressed to dyspnea, so additional radiographs were taken and revealed an air-filled esophagus and a well-defined soft tissue mass within the nasal passages. During endoscopy, the patient underwent cardiorespiratory arrest. On necropsy, a 1.5x1.5x1 cm, smooth, tan to white, semi-firm mass was found to originate from the soft palate. This mass had a 4.4x2.5x1.5 cm smooth, tan to white, semi-firm, pedunculated region that projected rostrally into the nasopharynx, which was presumed to be a large nasopharyngeal polyp. Approximately 90% of the nasopharynx was obstructed by the mass. A moderate amount of edema was within the caudal trachea and mainstem bronchi. Both right and left cranial lung lobes were mildly firm, but no obvious evidence of pneumonia was identified grossly. The cause of death was related to respiratory arrest due to nasopharyngeal obstruction by the large mass. On histopathology, the nasopharyngeal mass was characterized by proliferations of primitive cells, multifocally forming tubules and “glomeruloid” structures intermingled with areas composed of spindloid cells. The neoplastic cells were positive for Wilm’s Tumor on immunohistochemistry, and the mass was diagnosed as a nephroblastoma. The sections of lung revealed interstitial pneumonia. To the author’s knowledge, this is the first case of nephroblastoma developing in the nasopharynx in any animal species, and the first case of nephroblastoma described in a goat.

#### **SP-46: RIGHT VENTRICULAR EPITHELIOID HEMANGIOSARCOMA WITH PULMONARY METASTASIS IN A DOG**

Kaylin McNulty, Ryan Taylor, Wes Baumgartner

**Case Report:** An 11-year-old spayed female mixed breed dog presented for an acute hacking cough and increased respiratory effort, progressing to dyspnea. Thoracic radiographs revealed a diffuse nodular pulmonary pattern. The patient was euthanized. On necropsy the lungs had widely disseminated, raised, well-demarcated, tan to red nodules occasionally filled with blood. The liver was enlarged. Arising from the right ventricular free wall, extending into the lumen was an irregular, coarsely frondose, tan to red mass. On histopathology, neoplastic cells had a plump, epithelioid appearance with occasional cytoplasmic vacuoles. Cells frequently formed a sheet-like arrangement and occasional vague, thin channels toward the base. The cells were factor VIII positive.

The histological findings are most consistent with a hemangiosarcoma, epithelioid variant, with pulmonary metastasis and chronic passive congestion of the liver. To the authors' knowledge, this is the first report of a primary epithelioid hemangiosarcoma arising from the free wall of the right ventricle.

**Discussion:** Visceral hemangiosarcomas are most commonly found in the spleen and right atrium. The two variants are endothelial and epithelioid (histiocytoid). Cells of epithelioid variant are described as having an epithelioid appearance, moderate to abundant pale eosinophilic cytoplasm with occasional vacuoles, and rarely containing a single erythrocyte. Patterns may range from vasoformative slits, nests, or sheet-like arrangements. Locations of epithelioid hemangiosarcomas in the veterinary literature include the deep dermis, subcutis, skeletal muscle, gastrocnemius tendon and wide spread (myocardium, spleen, liver and lung). In this case, the histologic appearance and immunohistochemistry are most consistent with a hemangiosarcoma, epithelioid variant.

#### **SP-47: MOST FREQUENT INJURIES SUSTAINED BY FREE-RANGE CATS KILLED IN ROAD TRAFFIC ACCIDENTS IN ST. KITTS**

Samantha Zayas, Mary Anna Thrall, Anibal Armien, Pompei Bolfa

Worldwide, road transportation is a life-threatening risk for free-range cats. In developing countries, as St Kitts, where road conditions and cultural attitudes towards road use and animals differ, research characterizing injuries of cats involved in road traffic accidents (RTA) is limited. RTA-associated trauma is common among feline patients presenting for emergency veterinary care in St. Kitts; therefore, a cross-sectional study was performed to determine the prevalence of injuries in cats killed by RTA. Commuters were asked to call-in any dead cats seen on the roadside from January 2018 to August 2018. An investigator scanned and collected the cats and documented their geographical location. Microchipped and/or severely autolyzed animals were excluded. A necropsy was performed on each cat with gross lesions documented and photographed. Demographics (n=20) were evenly distributed between male and female cats, all intact and most less than one-year-old. Bone fractures were uncommon and mostly restricted to the mandible, maxilla, and pelvis. Visceral displacement were diaphragmatic herniation, abdominal eventration, and external abdominal hernia. The most common visceral injuries were multiple liver lacerations, single splenic lacerations, locally extensive pulmonary contusions, and traumatic proptosis. Subcutaneous hematomas were seen, however, usually associated with dermal abrasions or bone fractures. This study is intended to be a benchmark for veterinarians during their physical examination and initial diagnostic evaluation of the feline patient presenting with vehicular injuries. With the prevalence of RTA-associated injuries in free-range cats on St Kitts described, further studies can evaluate the medical approach towards such patients for a better prognosis.

#### **SP-48: DIAGNOSIS OF CANINE PERIARTICULAR HISTIOCYTIC SARCOMA BY SYNOVIAL FLUID CYTOLOGY**

Ying Ngo, Katie Boes, Sheryl Coutermarsh-Ott, Nikolaos Dervisis, Noelle Muro

A 7-year-old intact male Labrador retriever was presented for a 2 month history of acute onset right hindlimb lameness that failed to improve with medical management. On physical examination, the dog was Grade 4/5 lame and had stifle effusion in the same limb. Cytology of synovial fluid from the right stifle revealed 5,700 nucleated cells per microliter and 7% neoplastic round cells with vimentin-positive, CD18-positive, and cytokeratin-AE1/AE3-negative immunoreactivity compatible with periarticular histiocytic sarcoma (PAHS). Tissue biopsy with histopathology of the stifle swelling showed streams of infiltrative neoplastic spindle cells displaying positive immunoreactivity for CD18 and Iba-1 consistent with PAHS. There was no evidence of metastasis, and the dog's affected limb was amputated without complications. PAHS is a rare neoplasm of synovial macrophages (type A synoviocytes). While the patient's signalment and clinical presentation were typical of PAHS, this case is unique in that a diagnosis was made via synovial fluid cytology. Literature searches for malignant canine synovial fluid cytology revealed one metastatic transitional cell carcinoma and one metastatic bronchiolar-alveolar carcinoma. Although rarely reported, this case highlights the importance of considering neoplastic disease when performing a cytological evaluation of synovial fluid from a patient presented for lameness and joint pain.

#### **SP-49: ACROLEIN-INDUCED NASAL INFLAMMATION IN RATS**

Betsy Pray, Abigail Durkes

The nasal mucosa is the first area to be exposed to and thus damaged by a variety of inhaled toxins. One such toxin, acrolein, is a pervasive environmental pollutant. This reactive carbonyl is abundant in cigarette smoke, mobile exhaust, and industrial waste. More recently, acrolein has also gained attention as a biproduct of combustion of the "e-liquids" used in e-cigarettes. Though there are many clinical reports describing nasal irritation and congestion in people following acrolein exposure, there is limited literature on the in vivo effects of and relevant animal models for nasal mucosal tissue exposure to acrolein. In this study, Sprague-Dawley rats were placed in a 50L whole-body exposure chamber and exposed to either an experimental treatment of acrolein aerosolized in nitrogen (3 ppm acrolein with a total flow of 8 L/min) or a control treatment of filtered air. These treatments were performed for 5 hours per day, 5 days per week, and continued for 4 weeks total. Rats were sacrificed 4 hours after the last exposure, and tissues were collected immediately, fixed in formalin, and processed routinely with hematoxylin and eosin staining. Using of paraffin embedded nasal tissue, pathologic changes were characterized, including a mixed neutrophilic-lymphoplasmacytic infiltrate as well as hyperplasia and squamous metaplasia of the nasal epithelium. This study demonstrates that acrolein inhalation incites a significant inflammatory response in the nasal epithelium of rats.

#### **SP-50: ESTHESIONEUROBLASTOMA IN A CYNOMOLGUS MACAQUE**

Megan Zalek, Jennifer Lamoureux, Joseph Hampel, Keith Nelson

A three-year-old, intact male, Chinese-origin cynomolgus macaque presented for ptosis of the left eye (OS). Physical exam additionally revealed OS mydriasis, exophthalmos, abnormal retropulsion, and paralysis of the globe. OS direct pupillary reflex was absent, however, the consensual reflex of the right eye (OD) was normal. OD direct pupillary

reflex was normal, consensual reflex of OS was absent. OS external ophthalmoplegia was diagnosed. The eye was unresponsive to medical management and euthanasia was elected. Necropsy identified a pale-yellow discoloration of the left orbit, an indentation of the left temporal brain, and a focal bulge of the ventral aspect of the cranial vault. This was matched to a firm pale yellow to tan mass filling the left caudal sinuses and displacing turbinates and bone, including the orbit and ventral cranium. Histopathology revealed a highly cellular expansile neoplasm arising from and infiltrating the nasal turbinates and extending into the surrounding bone and skeletal muscle. The neoplasm was composed of spindloid to polygonal cells arranged in nests and packets separated by a fine fibrovascular stroma. These findings are most consistent with an esthesioneuroblastoma (also known as an olfactory neuroblastoma). Esthesioneuroblastomas are a malignant tumor of olfactory neural origin rarely diagnosed, but reported in humans, dogs, cats, horses, cows, mice, axolotls, and fish. This is only the second report of an esthesioneuroblastoma in a cynomolgus macaque.

#### **SP-51: CHRONIC EOSINOPHILIC GRANULOMATOUS PNEUMONIA IN A HORSE**

Kurt Williams, Elizabeth Carr, Eileen Henderson, Anna Gates

A 10 year old Andalusian gelding initially presented to the Michigan State University Veterinary Medical Center in September 2016 for dyspnea, lethargy, and nasal discharge that was unresponsive to antibiotic therapy, and mildly responsive to steroid and clenbuterol treatment. Biopsy of the lung at this time identified multifocal eosinophilic granulomas. Clinical signs progressed to respiratory distress, dyspnea, epistaxis and inability to work, leading to the euthanasia and necropsy of the horse in July 2018. Within the lungs, there were thousands of 0.1-0.3 cm firm, well-circumscribed, white to pale tan-green nodules scattered throughout the pulmonary parenchyma. Similar nodules were noted within the tracheobronchial lymph nodes, which were markedly enlarged; few similar nodules were present within the liver.

Histologically, the nodules in the lung varied from well circumscribed eosinophilic granulomas to foci of dense collagen with mild associated inflammation. Similar microscopic lesions were present in the tracheobronchial lymph nodes, and there was moderate eosinophilic inflammation in the mucosa of the large colon. A cause of the severe pulmonary inflammation was not identified. Eosinophilic pneumonia is uncommon in horses. Two eosinophilic inflammatory disorders, Equine Idiopathic Chronic Eosinophilic Pneumonia and Multisystemic Eosinophilic Epitheliotropic Disease have been described in horses affecting the lung. The cause and pathogenesis of these diseases, and their relationship to one another, is not known. The present case has clinical features and pathology similar to these two eosinophilic inflammatory disorders, suggesting shared pathogenesis between the two diseases.

#### **SP-52: TISSUE SPECIFIC EXPRESSION OF AQUAPORIN GENES IN RHIPICEPHALUS (BOOPHILUS) MICROPLUS**

Cecily Parell, Glen Scoles, Sara Davis

Vaccines established in the mid 21<sup>st</sup> century to control the spread of *Rhipicephalus microplus* and other cattle ticks have largely been unreliable for tick control and subsequent pathogen transmission due to sequence variations in the targeted antigen.

With acaricide resistance on the rise, better tick control methods are crucial for the preservation of global livestock production. Aquaporin, a transmembrane, osmoregulatory protein, has been identified as a potential antigenic target for a more effective vaccine due to its crucial role in successful tick feeding and reproduction, and its high sequence conservation. We hypothesized that Aquaporins are expressed in specific tick tissues. We investigated the pattern of expression of the Aquaporin genes of *R. microplus* in different tick stages and tissues via RNA isolation, subsequent cDNA synthesis, and quantitative real-time PCR. We discovered similar expression of Aquaporin 1 and 3 across life stages, while Aquaporin 2 is most greatly expressed in the ovaries of all tested life stages. This study represents one small part of a larger project underway in our lab to identify new target antigens for anti-tick vaccination. These findings suggest that use of Aquaporin 1 or 3 alone would not be as efficacious as antigenic targets due to the redundancy of expression. This data also corroborates previous published work that showed that the silencing of Aquaporin 2 significantly reduces tick fitness and larval survival rates. Tick population range expansion necessitates the development of alternative and more environmentally friendly methods for tick control. Anti-tick vaccination represents one promising, potential solution.

#### **SP-53: THERE IS MORE TO CDKN2A THAN MEETS THE EYE**

Sarah Zalar, Amanda Loftin, Mark Simpson, Jacqueline White, Colin McKerlie, Hibret Adissu

Cyclin dependent kinase inhibitor 2A (*Cdkn2a*) encodes proteins that regulate critical cell cycle regulatory pathways. We describe the histopathological findings in knockout *Cdkn2a* mice (*Cdkn2a*<sup>-/-</sup>, 2 males and 2 females) on a C57BL/6NCrl strain background produced by the International Mouse Phenotyping Consortium. Clinical phenotyping of *Cdkn2a*<sup>-/-</sup> mice (5 males and 5 females plus co-housed wild-type controls) showed abnormal eye morphology characterized by persistence of hyaloid vasculature, abnormal retinal pigmentation, abnormal lens morphology, and lens opacity (cataract). Histopathology identified a pigmented retrolental fibrous tissue along with posterior lenticonus secondary to lens capsule rupture, posterior subcapsular coagulation of lens proteins/Morgagnian globules (posterior subcapsular cataract), and focal retinal dysplasia. These retinal and lenticular changes were consistent with persistent hyperplastic tunica vasculosa lentis (PHTVL/PHPV) similar to those reported earlier in *Cdkn2a*<sup>-/-</sup> mice. Additional findings were small thymus with depleted medulla and cystic changes, decreased cortical thickness and cellularity in all four mice, and one of the two male mice had multifocal seminiferous atrophy. The findings in the thymus and testes were previously unreported and not predicted by clinical phenotype underscoring the discovery value of comprehensive histopathological analysis in phenotype characterization of mutant mice.

#### **SP-54: NECROPSY EXAMINATIONS OF LITTLE AUKS (ALLE ALLE) FROM A MASS STRANDING EVENT IN SCOTLAND**

Paul Smith, Adrian Philbey

**Background:** 56 Little Auk (*Alle alle*) specimens were submitted to the pathology department of the Royal (Dick) School of Veterinary Studies after a mass stranding

event on the East coast of Scotland in January 2016. This is the first monospecific necropsy investigation of this species.

**Objectives:** The aim of the project was to perform post mortem examinations on specimens to obtain data about their cause of death, health status prior to death and biometric data. The null hypotheses (Ho<sup>1</sup> & Ho<sup>2</sup>) were:

Ho<sup>1</sup> - There is no significant difference between the frequencies of each cause of death.

Ho<sup>2</sup> – There is no significant relationship between body weight and parasite burden.

**Methods:** Biometric data was recorded prior to necropsy. Standard avian necropsy techniques were used. Fecal samples were examined using microscopy to identify parasites. Parasites were cleared with lactophenol for Identification. Microscopic disease was cultured on MacConkey and horse blood agar.

**Results:** Granulomatous serositis and inanition were found commonly. *Contracaecum rudolphii* was the most prevalent parasite. A significant negative correlation between body weight and parasite burden was found. Inanition was significantly higher than the other causes of death.

**Conclusions:** *Contracaecum rudolphii* has only been recorded once before in *A. alle* 91 years ago. The biometric data identified the birds as the subspecies *A. a. alle* and allowed their likely natal origin of Svalbard/Greenland to be inferred. Finally, the causes of death were identified and showed inanition was significantly higher than the other causes of death, probably exacerbated by presence of parasites.

#### **SP-55: GINGIVAL HISTOPLASMOSIS: AN ATYPICAL PRESENTATION OF AFRICAN HISTOPLASMOSIS IN THREE BABOONS (PAPIO SPP)**

Taylor Johannigman, Olga Gonzalez, Shyamesh Kumar, Edward Dick Jr., John Dutton

**Background:** African histoplasmosis (*Histoplasma capsulatum* var. *duboisii*) usually presents as granulomatous skin and/or bone lesions. Gingival lesions as the sole manifestation of *H. capsulatum* var. *duboisii* have never been reported in baboons, and are a clinical diagnostic challenge.

**Methods:** Two female baboons, aged 2 and 7 years, presented with slightly raised linear to irregular, occasionally ulcerated, multinodular lesions of the gingiva. The 2-year-old animal was euthanized due to poor long term prognosis. Lesions were first noted at necropsy in the 7-year-old animal. Both animals were euthanized, tissues were collected, processed, and evaluated by pathologists. Similar gingival lesions were noted clinically in an 11-year-old female baboon and a biopsy was submitted. The animal was later euthanized, but lost to follow-up.

**Results:** Histopathology identified multifocal to coalescing, large nodular aggregates of numerous histiocytes and multinucleate giant cells with myriads of intra-histiocytic and extracellular yeasts, approximately 3-10 microns in diameter, with a 2-micron thick capsule and a nucleus (morphologically consistent with *Histoplasma capsulatum* var.

*duboisii*). In one case, the granulomatous inflammation infiltrated the alveolar bone. No other areas on the body were affected.

**Conclusions:** This is the first report of primary gingival histoplasmosis in captive baboons similar to some human presentations with African histoplasmosis. Grossly, lesions can be indistinguishable from other more common disease processes such as bacterial gingivitis or gingival hyperplasia. Clinical outcomes of primary gingival histoplasmosis in baboons are unknown, and diagnosis of this disease may further complicate colony management decisions.

#### **SP-56: ASSOCIATION OF CANINE MALIGNANCY WITH COMPLETE BLOOD COUNT AND SERUM BIOCHEMISTRY ABNORMALITIES**

Damali Zakers, Britton Grasperge

With the difficulty of diagnosing malignancies in veterinary medicine, additional clinical signs are very helpful. Several hematologic and serum biochemical abnormalities have been associated with specific malignancies, such as hemophagocytic histiocytic sarcoma in dogs, which shows a pattern of hypoalbuminemia and hypocholesterolemia. The purpose of this study is to find additional malignancies that are likely to have their own characteristic patterns. This can lead to early recognition and a more complete differential diagnoses list in patients. A combined retrospective and prospective study was performed. Patients seen from August 2013 to June 2018 were reviewed for their initial pretreatment complete blood counts and biochemistry profiles. At least 20 patients for each neoplasm: histiocytic sarcoma, mast cell tumor, apocrine gland anal sac adenocarcinoma, and osteosarcoma were required for inclusion in the study. All four neoplasms showed significant clinicopathologic manifestations.

Histiocytic sarcoma showed a positive correlation with values attributable to a regenerative anemia which is commonly reported with the hemophagocytic variant of the disease. Apocrine gland anal sac adenocarcinoma displayed a positive correlation with hypercalcemia.

Osteosarcoma showed no significant correlation. Mast cell tumors reflected correlation with the leukocytes, specifically displaying a typical stress leukogram. Inclusion of additional tumors and greater numbers of animals will help to expand our ability to predict neoplasms with less invasive procedures.

#### **SP-57: SEPTICEMIC LISTERIOSIS IN A COLONY OF SUGAR GLIDERS**

Amber Olson, Annie Zimmerman, Dodd Sledge

A privately-owned colony of sugar gliders experienced 4 unexpected mortalities in adult animals over 2 weeks. Some animals had no clinical signs prior to death, but others had a 1 to 2-day history of lethargy and anorexia. Full necropsies were performed on three of these animals. In the animal that had the least amount of autolysis, the diaphragmatic surfaces of the caudal lung lobes were covered by tan mats of fibrin and there were approximately twenty 0.5mm-1mm diameter round, tan foci randomly scattered throughout the hepatic parenchyma. Microscopically, the visceral pleura and epicardium

were markedly expanded by large numbers of neutrophils, abundant fibrin, and necrotic debris. The adventitia, submucosa, and tunica muscularis of the esophagus were expanded to effaced by large numbers of neutrophils and necrotic debris. The liver and splenic white pulp contained multifocal areas of necrosis characterized by variably-sized accumulations of karyorrhectic debris, neutrophils, and extracellular coccobacilli bacteria. *Listeria monocytogenes* was cultured from the liver and lung, and there was immunohistochemical labeling for listeria antigen in affected areas of lung, pericardium, esophagus, liver, and spleen. Listeriosis affects a wide variety of species and can present as reproductive failure, neurologic disease, or septicemia. This represents a case of septicemic listeriosis. Based on the diet of these animals, which included melons, and the severe esophageal lesions, contaminated food represents a likely source of infection in this colony of sugar gliders.

#### **SP-58: MALIGNANT CATARRHAL FEVER-LIKE DISEASE DUE TO NATURALLY-OCCURRING OVINE HERPESVIRUS 2 INFECTION IN A JUVENILE EWE**

Jonathan Miller, Kelly Ramsay, Chrissy Eckstrand

The gammaherpesvirus ovine herpesvirus 2 (OvHV-2) is the causative agent of sheep-associated malignant catarrhal fever (MCF), a severe and commonly fatal disease syndrome in non-adapted ungulates such as cattle and bison. Domestic sheep serve as the natural reservoir for OvHV-2 and are thought to carry the virus asymptotically, though MCF-like clinical disease in sheep has been rarely described. Here, we present the pathological findings in a juvenile ewe naturally infected with OvHV-2 exhibiting MCF-like disease. The ewe died following a 1-day history of seizures, diarrhea, anorexia, and blindness. Gross findings from necropsy were unremarkable except for mild pleural effusion. Histological examination revealed severe multiorgan lymphoplasmacytic-histiocytic vasculitis. Neurological signs were attributed to moderate multifocal lymphoplasmacytic-histiocytic meningoencephalitis, likely related to systemic vasculitis. Real time PCR detected OvHV-2 DNA in lymph node tissue and affected large arteries. *In situ* hybridization was performed to further localize the virus and demonstrated large amounts of OvHV-2-specific nucleic acid in inflamed vessels. The sheep was negative for pestiviruses, bluetongue virus, epizootic hemorrhagic disease virus, and small ruminant lentivirus; had hepatic trace mineral levels (including selenium) within reference ranges, and aerobic culture of lung yielded no bacterial growth. Previous reports indicate that the development of MCF-like lesions in sheep are typically associated with underlying stressors, concurrent disease, or nutritional deficiencies. Although no nutritional deficiency or concurrent significant disease was identified in this animal, further investigation revealed ongoing problems with caseous lymphadenitis, internal parasitism and mineral deficiencies in the herd of origin.

#### **SP-59: FINDING THE SOLUTION TO CALCIUM OXALATE UROLITHIASIS: A COMPARISON OF THE DISSOLUTION ABILITIES OF CITRATES AND ORGANIC ACIDS**

Samuel Bournier-Drawbridge, Balazs Szladovits

Treatment of calcium stone disease through dissolution therapy has not been achieved due to a lack of known appropriate dissolution agents. This study aimed to compare

different citrates and organic acids, determining the extent to which they would dissolve calcium oxalate dihydrate (COD) crystals *in vitro*, a common constituent of calcium uroliths. COD crystals were treated with sodium EDTA (positive control), deionised water (negative control), sodium chloride, sodium citrate, potassium citrate, citric acid, lactic acid, tartaric acid, phosphoric acid and acetic acid, at a final concentration of 0.15M. These agents were added to a counting slide containing the COD crystal solution and 5-10 high power field images were taken at specific time intervals. This was used to qualitatively observe effect of these agents on the crystals, as well as semi-quantitatively measure changes in number, size and mass. It was found that sodium citrate, potassium citrate and phosphoric acid had the best dissolution activity. There was a significant reduction in crystal number at 30 minutes (100% for the citrates, 70% for phosphoric acid), crystal size (80% for sodium citrate and phosphoric acid, 61% for potassium citrate) and mass at 5 minutes (80% for phosphoric acid, 76% for sodium citrate and 53% for potassium citrate). Tartaric and lactic acid had moderate and sodium chloride and acetic acid had the lowest dissolution activity. These data show citrates and phosphoric acid to have the best ability to dissolve COD crystals, therefore the recommended agents for further COD containing urolith dissolution studies.

#### **SP-60: MORPHOLOGICAL CHARACTERIZATION OF THE ADHERENCE AND INVASION OF STREPTOCOCCUS AGALACTIAE TO THE INTESTINAL MUCOSA OF TILAPIA OREOCHROMIS SP. IN VITRO MODEL**

Gersson Vásquez, Carlos Iregui, Paola Barato

**Introduction:** Streptococcosis caused by *Streptococcus agalactiae* is considered the main bacterial disease in tilapia. In Colombia and the world. In the present investigation we characterize morphologically the first stages of interaction of *Streptococcus agalactiae* with the intestinal mucosa of tilapia using an *in vitro* organ culture of tilapia intestine.

**Materials and Methods:** Seventy-two explants were infected with three concentrations of bacteria and three incubation times. Eighteen explants were considered as negative control. Light and transmission electron microscopy, immunohistochemistry, Toluidine blue and PAS-Alcian blue stains were performed.

**Results:** An *in vitro* culture of tilapia intestine was standardized. We showed that *Streptococcus agalactiae* sheds off its capsule. The bacterium adheres as a single individuum, in groups, or in chains and is able to divide on the apical border of enterocytes. It adheres and invades through the apical portion of the intestinal folds, using the transepithelial route. Once within the cytoplasm of enterocytes, the bacteria continue to divide. The microorganisms leave the cells to reach the propria and travel through the circulation. No evidence of an immunoinflammatory reaction or goblet cells response was seen during the process of adherence and invasion of the pathogen.

**Conclusions:** *Streptococcus agalactiae* of tilapia in Colombia adheres in groups, chains or solitary bacteria. It is divided on the apical border of the enterocytes, adheres and invades the apical portion of the folds. It crosses the mucosa using the transcellular route and no response of lymphocytes or goblet cells was observed.

## **SP-61: AN OUTBREAK OF METABOLIC BONE DISEASE IN HERON AND EGRET CHICKS AT A WILDLIFE REHABILITATION CENTER IN NORTHERN CALIFORNIA**

Molly Horgan, Brian Murphy, Rebecca Duerr

An increase in cases of metabolic bone disease (MBD) in 6 species of heron and egret chicks (family Ardeidae) was identified at a wildlife rehabilitation center in Fairfield, CA between April and August 2018. 130/410 (32%) birds had clinical evidence of MBD compared to 40/637 (6%) during the same period of time in 2017. Cases were diagnosed either at intake or after days-weeks in care, and were characterized by fractures, deformities and increased flexibility of multiple long bones. Necropsies were conducted on 146 nestlings and fledglings that died or were euthanized either due to MBD or for unrelated conditions. Histologic findings were consistent with multiple MBD subtypes and were characterized by elongation of the physis, retention of cartilage cores in the primary spongiosa, thinning/lack of diaphyseal cortical bone compaction, and folding fractures. The diameter of the parathyroid gland of birds diagnosed with MBD in care was significantly larger than that of unaffected birds ( $p=.0001$ ). A dietary deficiency of vitamin D was proposed as the cause of the MBD and staff began to supplement every bird's diet with 3240 IU/kg/day oral Vitamin D3 in early July 2018, after which the number of birds developing MBD in care declined to a level similar to previous years (3/84, 3.6%). Relatively little has been reported in the peer reviewed literature on MBD in wild birds even though it is a common problem in wildlife rehabilitation. This study characterizes the clinical, gross, radiographic and histologic features of MBD in young herons and egrets.

## **SP-62: A COMPARISON OF BOVINE AUTOPSIES USING DIGITAL IMAGES VERSUS STANDARD METHODS**

Rachel Redick, Jamie Rothenburger, Cynddae McGowan, Sylvia Checkley, Eugene Janzen

The number of cattle and other large animals submitted to diagnostic laboratories can be limited by factors such as geographical distances and expense. In response to these challenges, a growing number of cattle practices encourage their clients to use digital images to collect autopsy information. Trained individuals complete a standard dissection, collect a prescribed set of images of major organs and lesions, and then send these to veterinarians for diagnosis. Although widely-used, the validity of substituting digital images for traditional diagnostic assessments is unknown. The objective of this study was to compare the accuracy of digital images to standard pathology diagnostic procedures. Between 2014 and 2017, 225 cattle were submitted to the University of Calgary's Diagnostic Services Unit where veterinary pathologists conducted autopsies, histopathology and other required ancillary tests to reach a diagnosis. During autopsies, they also collected standardized images that were then sent to a veterinarian panel for diagnostic interpretation. We will use Cohen's kappa coefficient to assess for agreement between the panelists' and veterinary pathologist's conclusions and identify patterns of agreement/disagreement among lesion types. Understanding the strengths and weaknesses of digital imaging in field autopsy diagnosis will provide valuable information to veterinarians in the use of this technique.

Image autopsy techniques could provide a better understanding of diseases that affect production animals and contribute significantly to animal health intelligence networks. It may also be applicable to wildlife disease monitoring and developing countries as both a diagnostic technique and to address food safety issues.

### **SP-63: CAUSES OF MORBIDITY AND DEATH IN SPRAGUE-DAWLEY AND WISTAR RATS IN 2-YEAR CARCINOGENICITY STUDIES**

Nicholas Vetter, Charissa Dean, Keith Nelson

Determination of cause of morbidity and death (COD) in carcinogenicity study animals can aid in understanding both test article related and background pathology within test animal populations. Examination of COD across gender and strain may reveal pathological tendencies within these groups, differences between them, and how these change over time, assisting in the evaluation of potential toxicity. We examined data from 54 carcinogenicity studies performed at a single contract research organization from 2006 through May 2018 utilizing 31,719 rats (28,038 Sprague-Dawley and 3,681 Wistar, approximately equal numbers of each sex) to survey COD variance between rat strains. COD determination only reflects animals that died or were euthanized during the course of the study prior to scheduled termination. Among Sprague-Dawley rats which died on study (60.8% of males, 61.6 % of females), the most common COD was pituitary tumor (34.7% in males, 53.0% in females) followed by undetermined cause (24.2%) in males and mammary tumor (26.7%) in females. Among Wistar rats which died (21.2% of males, 27.6% of females), pituitary tumor was the most common COD (28.7% in males, 36.8% in females), followed by liver tumor (9.2%) in males and mammary tumor (10.0%) in females. With a robust base of study data, this survey provides substantial insight into the prevalent causes of death in two commonly used rat carcinogenicity model strains. Future work will focus on trends in COD over time and their potential relationship to breeding, strain development, changes in husbandry, changes in humane endpoint guidelines, and other factors.

### **SP-64: RENAL METASTASES OF MAMMARY CARCINOMA IN AN INTACT MALE CANINE**

Frances Fan, Vanessa Oakes, Tanya LeRoith, Sheryl Coutermarsh-Ott

A seven-year-old, mixed-breed, intact cryptorchid male canine presented for castration. On physical exam, a soft, papilliferous, and pedunculated skin mass was identified on the right ventral caudal thorax. The mass was surgically removed and submitted for histopathology. Microscopically, the mass was composed of an infiltrative epithelial neoplasm on a scirrhous stroma. Neoplastic cells often formed acini and had indistinct cell borders, small to moderate amounts of eosinophilic cytoplasm, and polygonal nuclei. Adjacent to the neoplasm were hyperplastic and dysplastic mammary glands surrounding a teat duct. The cutaneous neoplasm was consistent with a mammary carcinoma. Estrogen-secreting Sertoli cell tumors have been linked to some mammary tumors; unfortunately, histopathology of the cryptorchid testicle was not performed. Post-surgery, the dog was slow to recover and expired within 48 hours. Necropsy revealed multiple small firm white foci within both renal cortices in addition to bilateral adrenal cortical hyperplasia, *Dirofilaria* organisms in the right atrium and descending

vena cava, and chronic bronchitis. The probable renal, cardiac, and pulmonary compromise is consistent with the dog's poor anesthetic recovery. The renal cortical masses, which effaced renal tubular and glomerular structures, histologically resembled the mammary carcinoma excised from the thorax. Neoplastic cells were embedded in a scirrhous stroma, formed acini, and were cuboidal to attenuated, with distinct borders and variable amounts of eosinophilic cytoplasm. Collectively, these findings support a diagnosis of metastatic mammary carcinoma. Mammary tumors of any type are uncommon in the male dog; metastatic mammary carcinoma has not been previously described.

#### **SP-65: EFFECT OF CRANBERRY EXTRACT ON FELINE ORAL SQUAMOUS CELL CARCINOMA PROLIFERATION AND MIGRATION IN VITRO**

Erika Pugh, Haili Wang, Jason McCallum, Chelsea Martin

Feline oral squamous cell carcinoma (FOSCC) is the most common oral malignancy in cats and is known for treatment resistance and poor prognosis. Several types of berries contain a variety of bioactive phytochemicals, such as anthocyanins, that have demonstrated anticancer activities in human cancer studies. An *in vitro* viability assay (MTT) was used to determine the effect of crude cranberry, blueberry, and blackberry extracts on FOSCC cells (SCCF2). At 83 µg/ml, crude cranberry extract (CCE) exhibited the greatest reduction in FOSCC viability (43%), compared to blueberry (31%) and blackberry (16%) extracts ( $p < 0.05$ , ANOVA). CCE was divided into 5 fractions, with the anthocyanin-enriched fraction and the flavonol glycoside-enriched fraction inhibiting SCCF2 viability to the greatest degree (68% and 90%, respectively). A scratch assay was used to determine the effect of CCE on migration of three FOSCC cell lines (SCCF1, SCCF2, SCCF3). Preliminary results indicate migration was slowed by 50 µg/ml and 71 µg/ml CCE in all three cell lines, with evidence of cell death at 71 µg/ml. Migration of SCCF1 cells was also reduced by 25 µg/ml of CCE. These findings demonstrate that crude cranberry extract has the greatest activity against SCCF2 viability compared to crude blueberry and blackberry extracts, which may be due to anthocyanins and flavonol glycosides, but additional characterization is required in order to identify the responsible phytochemicals. The effect of CCE on SCCF2 migration appears to be cell-line specific. Future experiments will combine cranberry extracts with conventional chemotherapy drugs to determine if FOSCC chemosensitivity can be improved.

#### **SP-66: PROBIOTIC SUPPLEMENTATION OF ANTIRETROVIRAL THERAPY IMPROVES GASTROINTESTINAL IMMUNITY IN SIMIAN IMMUNODEFICIENCY AND HUMAN IMMUNODEFICIENCY VIRAL INFECTIONS**

Amanda Loftin, Carol Vinton, Jason Brenchley

Simian Immunodeficiency Virus (SIV) infection of Asian macaques recapitulates key pathological features of Human Immunodeficiency Virus Type 1 (HIV) within the gastrointestinal tract of individuals. Studies in SIV-infected macaques support a model of gut epithelial tissue damage and disruption of gastrointestinal homeostasis. This dysbiosis permits microbial translocation into systemic circulation leading to persistent immune dysfunction despite effective suppression of plasma viral load with antiretroviral

therapy (ART). This leaves HIV-infected, ART-treated individuals at increased risk of morbidities and mortalities such as cardiovascular disease. We previously demonstrated oral probiotics promote increased intestinal CD4+ T-cell reconstitution during ART treatment in SIV-infected macaques, suggestive of immunological benefit. Our current objective is to investigate probiotic supplementation in ART-treated patients utilizing a novel cross-translational model of HIV and SIV infected human and non-human primates. This phase II study evaluated probiotic mediated effects on gastrointestinal CD4+ T-cell reconstitution in HIV-infected, ART-treated individuals using Visbiome Extra Strength probiotic. Serum and colonic biopsies were collected at entry and after 24-weeks of intervention. Rectal biopsies were stained against CD4 and CD68/CD163. The results showed increased CD4+ T-cell density in colonic lamina propria of Visbiome treated participants compared to baseline. Visbiome Extra Strength administration also increased MPO staining, a marker for neutrophil infiltration and showed a trend toward decreased plasma levels of D-dimers, an inflammatory biomarker. Elevated D-dimers are associated with cardiovascular disease in ART-treated, HIV-infected individuals. In summary, we demonstrate probiotic supplementation in HIV-infected, ART-treated individuals enhances gastrointestinal immune function through increasing reconstitution of CD4+ T cells in colonic epithelium.

#### **SP-67: DETECTING CANINE RETICULOCYTES AND POLYCHROMATOPHILS USING U-NET NEURAL NETWORK ARCHITECTURE**

Leanne Smaller, Cosmin Stamate, Adam Foster, Balázs Szladovits

Since surpassing human technicians in image classification performance during the 2012 ImageNet competition, neural networks have proved invaluable to the medical diagnostic toolbox. Similar techniques in veterinary medicine are not yet widely adopted and tasks such as cell counting are still commonly performed by laboratory technicians, which are time-consuming, and carry the potential for human error.

We investigated using neural networks to predict reticulocyte percentage determination and semi-quantitative grading of polychromasia for the assessment of regeneration of anaemia. Multiple images were taken of both the new methylene blue and Wright's stained blood smears for a dataset consisting of 79 blood smears from dogs diagnosed with variable degrees of anaemia at the Royal Veterinary College between December 2017 and March 2018. A baseline of 339 images was manually annotated to identify red blood cells, polychromatophils and reticulocytes. This dataset was used to train a deep convolutional neural network, using U-Net architecture.

On a randomly selected 240 fields, the sensitivity, specificity and positive predictive value for reticulocytes, was 85%, 98% and 74% and for polychromatophils 89%, 99% and 71%, respectively. The most common reasons for reticulocyte false positives were erroneous identification of punctate cells, poor staining or poor quality images. The latter two reasons were also explanations for polychromatophil over-prediction.

The promising results support further investigation into the performance of the neural network. Further improvement of the results can be conducted by using annotation from

more than one technician, catering for subjectivity and increasing the overall image database for testing.

### **SP-68: INTERSEX FISH HISTOPATHOLOGY IN THE UPPER TENNESSEE RIVER SYSTEM**

Chelsea Hester

Intersex fish are a growing concern to scientists studying the health of aquatic ecosystems. Intersex condition in fishes has been caused by endocrine disrupting chemicals (EDCs) stemming from treated wastewater effluents, which are postulated to cause population collapses and lower reproductive success in gonochoristic (fixed sex) species. Therefore, the use of gonochoristic fish as an indicator species of EDCs has flourished. Efforts to detect and quantify intersex presence and severity are increasing in the field of pathology. The aim of this study is to assess the presence and severity of oocytes, utilizing histopathology, in the testes of fishes inhabiting the Upper Tennessee River drainage system. Gonad samples from 143 fish representing 4 species (Whitetail Shiner *Cyprinella galactura* and Spotfin Shiner *C. spiloptera*, Central Stonerollers *Camptostoma anomalum*, and Smallmouth Bass *Micropterus dolomieu*) were collected and assessed for intersex condition and severity. A modified intersex severity scoring system was developed for this study to account for spatial arrangement of oocytes in the gonad. The scoring system was grade 0 (not remarkable) to grade 4 (severe) based on gonad ova field percent coverage. Results indicated low frequencies of intersex (14% intersex) and grade 1 to 2 (minimal to mild) intersex severity. Therefore, the rivers in Tennessee are less likely to be affected by EDCs when compared to other aquatic ecosystems in previous studies containing large quantities of intersex fish occupying severely degraded environments.

### **SP-69: NEURONAL CEROID LIPOFUSCINOSIS IN A DOMESTIC BLACK AUSTRALORP CHICKEN**

Helena Vogel, Andrew Cartoceti, Kevin Woolard

A 1 year old female black australorp chicken presented for necropsy with a history of progressive paraplegia. Necropsy examination observed no gross abnormalities. Histologically, upper motor neurons in the brainstem showed marked cytoplasmic vacuolation and chromatolysis, with accumulation of yellow-brown, granular cytoplasmic material that displaced Nissl substance and nuclei. Lesions were restricted to the brainstem, with sparing of cerebellar Purkinje cells. Neuronal cytoplasmic accumulations were autofluorescent, positive for periodic acid-Schiff (PAS), and Luxol fast blue (LFB). Immunohistochemistry for BiP showed upregulation of the ER stress response, a common mediator of neuronal damage in mammals and birds. The morphological and histologic properties of this case are consistent with the diagnosis of neuronal ceroid lipofuscinosis (NCL) and presents a new uncharacterized species with an unusual distribution of neuronal damage for NCL. In contrast to most cases of NCL in domestic animals, the Purkinje cells and cerebellum in this patient were unaffected, with disease limited to brainstem nuclei.

### **SP-70: ESTHESIONEUROBLASTOMA IN A HORSE: HISTOLOGIC, IMMUNOHISTOCHEMICAL, AND ULTRASTRUCTURAL CHARACTERIZATION**

Juan Pablo Velasco Montes de Oca, Beatriz Vanda Cantón, Danilo Méndez Medina, Mireya Juárez Ramírez, Guillermo Corredor Alonso, Mariano Hernández, Elena García

A 14-year-old mare with ataxia, left exophthalmia and epistaxis presented an ethmoidal hematoma, diagnosed by biopsy. Two weeks later, a radiographic study showed a mass that occupied a large part of the ethmoidal cavity. The mare underwent euthanasia and postmortem examination. During gross examination, on the left sagittal cut surface of the head, rostral to the cranial cavity and extending towards the frontal and sphenopalatine sinus, there was a 13 cm well-demarcated mass, which on cut section had a reddish brown-fleshy appearance, with a soft consistency. Histologically, the nasal epithelium was elevated by a well-defined and highly cellular neoplasm, consisting of polygonal cells arranged in bundles and cords. The tumor cells had indistinct borders, moderate amount of fine granular pale eosinophilic cytoplasm and a round to oval nuclei with coarsely clumped chromatin. Mitosis were rare and there were some foci of necrosis. The intracytoplasmic granules showed argyrophilic positivity with Churukian Schenk stain in several areas of the neoplasm. Multifocally, the cytoplasm of tumor cells expressed strong positivity to Synaptophysin and Chromogranin-A. Ultrastructurally, the neoplasm showed polygonal and elongated cells with cytoplasmic processes forming empty lumens or with filaments. The nuclei were round to oval; in the cytoplasm, there was moderate to abundant glycogen, some mitochondria, endoplasmic reticulum and vesicles. Some filaments and microtubules with small electron dense granules (<100nm) were found. Intercellular junctions were not identified. Neurofilaments identified by electron microscopy, supported by the findings in gross and light microscopy was the diagnostic criteria for the diagnosis of this neoplasm.

### **SP-71: RADIOTHERAPY RESPONSIVE FELINE PROGRESSIVE CUTANEOUS DENDRITIC HISTIOCYTOSIS**

Rebecca Tierce, Tiffany Wormhoudt, Christine Olver, Susan LaRue, Mary-Keara Boss

Histiocytic proliferative disorders commonly occur in dogs and less frequently cats. Feline progressive histiocytosis (FPH) is a rare, initially indolent cutaneous neoplasm that progresses to affect lymph nodes and internal organs in terminal stages. FPH has a poor long-term prognosis since all previously documented treatments, including chemotherapy and immunomodulatory therapy, lack efficacy. Here we report the first case of radiotherapy responsive FPH. A 12-year-old neutered domestic medium hair initially presented for an enlarged submandibular lymph node that was excised with an adjacent intradermal mass. Histopathology revealed a poorly circumscribed, nonencapsulated dermal nodular aggregate of densely packed, randomly arranged cells consistent with lymphohistiocytic proliferation. A presumptive diagnosis of FPH was made based on characteristic cellular features including indistinct borders, eosinophilic cytoplasm, and round-to-oval or folded nuclei with finely stippled chromatin and admixed neutrophils and lymphocytes. Postsurgical recurrence occurred and continued to progress despite prednisolone trials. Ten months later, on presentation to Colorado State University's Veterinary Teaching Hospital, fine needle aspirate was performed and cytology results were consistent with the previous biopsy. Computed Tomography (CT)

was completed for radiation planning purposes. On CT scan, the submandibular 2.2x2.6x1.7 cm mass was representative of known FPH and targeted with external beam, stereotactic radiotherapy with 24 Gy administered in three 8 Gy fractionations over consecutive days. The tumor rapidly decreased in size and remained reduced until the patient was euthanized for unrelated reasons. This case characterizes a rare cutaneous neoplasm and to our knowledge documents the first instance of FPH responding to therapy.

#### **SP-72: THREE CASES OF CARCINOMA IN CHRYSOPELEA PARADISI**

Ashley Saver, Vanessa Oakes, Tanya LeRoith, D. Sponenberg, Calvin Lau

Within one week, three mature adult female paradise flying snakes (*Chrysopelea paradisi*) from the same research colony at Virginia Tech presented to the Virginia-Maryland College of Veterinary Medicine necropsy service. The colony was composed of fourteen wild-caught snakes that have lived in captivity for several years. Two out of three snakes were found dead in their cage, while the third was unable to be resuscitated after becoming unresponsive. Upon necropsy, all three had hepatic masses and subsequent histopathological examination revealed carcinoma. Differentials for the etiology of a high incidence of neoplasia in a captive snake population include carcinogen exposure, viral infection, chronic hepatitis, and genetic predisposition. Histologically, there was no evidence of viral inclusions or chronic inflammation and the snakes were not exposed to any known carcinogens during their time in the research colony. Thus, it is likely that these hepatic masses are the result of a genetic predisposition. Colubrids, compared to other families of snakes, are particularly prone to developing proliferative hepatic lesions that progress to carcinoma with time. Neoplasia is more commonly reported in captive snakes because they tend to have an increased lifespan due to good husbandry, stable environmental conditions, a lack of predators, and access to prompt veterinary care and diagnostics.

#### **SP-73: CHARACTERIZATION OF BALLOONING DEGENERATION IN MOUSE MODELS OF NONALCOHOLIC STEATOHEPATITIS**

India Napier, May Liu, Jean-Rene Galarneau, Keith Mansfield, Chandra Saravanan

Animal models are required to gain insights into the pathogenesis of nonalcoholic steatohepatitis (NASH) and evaluate efficacy and/or safety of potential drug candidates targeting NASH. However, none of the available models fully reflect the clinico-morphological patterns of human NASH. Particularly, hepatocellular ballooning degeneration (BD), a key morphological feature in human NASH, was not recognized in mouse models. Literature suggests that sonic hedgehog (shh) is a valuable marker to identify BD in human NASH liver. The goal of the study was to examine whether shh expression is a molecular correlate for BD in NASH animal models as shown in human NASH. Three dietary and/or genetic mouse models of NASH were used, namely high fat diet fed (HFD) Lepob/ob (leptin deficient), KKAy, and C57BL/6J models. H&E evaluation of liver from these models showed steatosis with variable degrees of lobular inflammation and pericellular fibrosis, but not BD. Affymetrix-based transcriptomics did not show an increase in shh mRNA signals in both Lepob/ob and C57BL/6J models. This data was confirmed by in situ hybridization experiments with lack of shh mRNA

upregulation in liver of all three models. Whereas, shh protein was upregulated and localized in few ballooned and non-ballooned hepatocytes and cholangiocytes of human NASH liver. In summary, in addition to lack of morphologic evidences of BD, shh expression as a molecular correlate for BD was absent in mouse models of NASH. Further characterization of hepatocellular BD in human NASH may help in the study of NASH pathogenesis and its reverse translation to animal models.

#### **SP-74: EVALUATION OF TIME AND TEMPERATURE ON FELINE BRONCHOALVEOLAR LAVAGE FLUID STORED IN EDTA**

Mackenzie Walker, Susan Fielder, Laura Nafe, Jared Taylor

**Background:** Bronchoalveolar lavage fluid (BALF) is an important diagnostic specimen in feline respiratory disease. Previous studies show that storage conditions affect cytologic analysis and subsequent diagnosis, but these did not address samples stored in EDTA.

**Objectives:** Evaluate effects of storage conditions on cytologic evaluation of BALF in EDTA

**Methods:** BALF was collected from 32 cats in another research project. Samples were divided into five aliquots and placed in EDTA. One aliquot was processed within one hour. Four aliquots were stored at 24°C (room temperature) and 4°C (refrigerator) and processed at 24 h and 48 h. A 300 differential cell count (DCC) (neutrophils, eosinophils, and mononuclear cells) and cell morphology score were performed on a Wright's stained cytospin preparation of all samples.

**Results:** Eosinophil percentage ( $p < 0.0001$ ) and cell morphology score ( $p = 0.002$ ) in BALF stored at room temperature for 48 h were significantly different from baseline. No difference was found in eosinophil percentages or cell morphology score in samples stored at other conditions. No difference was found in neutrophil percentages.

**Conclusion:** Changes in eosinophil percentages and cellular morphology were identified only in feline BALFs in EDTA stored at room temperature for 48 h. Fewer changes were seen in DCC and cell morphology in samples stored in EDTA compared to previous studies with samples stored without additive. BALF submitted for cytologic evaluation should be submitted in EDTA and analysis performed promptly to improve cytologic evaluation and interpretation.

#### **SP-75: DOES SIZE MATTER? REDUCED PROSTATE SIZE ALTERS URINARY FUNCTION IN MALE MICE**

Hannah Ruetten, Helen Zhang, Kyle Wegner, Peiqing Wang, Zunyi Wang, Dale Bjorling, William Ricke, Paul Marker, Chad Vezina

Our current understanding of lower urinary tract dysfunction (LUTD) in intact male dogs typically attributes dysfunction to an enlarged prostate gland. Studies have clinically correlated benign prostatic enlargement with LUTD, but mice have differing prostatic

anatomy and the relationship of prostate size and urinary function has not been explored in mice.

We reduced prostate size using three different methods, surgical castration, finasteride treatment, and genetic modification. All mice were on a C57BL/6J background and each group had six to nine mice. Cystometry (CMG) and spontaneous void spot assays (VSA) were used to assess urinary function. Mouse urinary function was assessed, mice were humanely euthanized, and tissues collected at nine weeks of age.

Castration reduces body, prostate, seminal vesicle, and bladder mass compared to sham castration ( $p=0.0019$ ,  $<0.0001$ ,  $<0.0001$ ,  $0.019$ ). In castrated mice, CMG revealed decreased void duration, voided volume, and baseline bladder pressure paired with increased urine area on VSA ( $p=0.0154$ ,  $0.0494$ ,  $0.004$ ,  $0.0301$ ). Finasteride treatment reduces prostate and seminal vesicle mass compared to controls ( $p=0.0009$ ,  $<0.0001$ ). CMG revealed an increase in time between voids and voided volume ( $p=0.0394$ ,  $0.0168$ ) and VSA detected no difference between groups. Genetically modified mice collection is ongoing.

These preliminary results indicate that reduction of prostate size alters urinary function in male mice. The culminated results of this study will inform researchers on the utility of altering mouse prostate as a means to study canine LUTD, and help tailor future studies to make better use of prostatic LUTD mouse models.

#### **SP-76: FIRST REPORT OF CUTANEOUS EPITHELIOTROPIC LYMPHOMA IN A NON-HUMAN PRIMATE (BABOON; PAPIO SPP.)**

Elvira Carias, Megan DeLorenzo, Olga Gonzalez, Shyamesh Kumar, Edward Dick Jr

**Background:** Cutaneous epitheliotropic lymphoma has not been previously reported in non-human primates.

**Methods:** A 9 year old baboon was presented with pruritic lesions on the right forearm. Biopsy was performed and skin samples were submitted for evaluation. Additional sections were prepared for immunohistochemical labeling for CD3, CD4, CD8, CD20, and CD68 markers using standardized protocols. Paraffin embedded tissue scrolls were submitted for qualitative QPCR for detection of STLV and HTLV viruses.

**Results:** Histologically, the dermis, adnexal structures and perivascular spaces were expanded and replaced by a poorly demarcated, infiltrative neoplasm composed of sheets of round cells consistent with intermediate-sized lymphocytes. There were scattered eosinophils, macrophages, and rare multinucleated giant cells. Neoplastic cells infiltrated into the epidermis and adnexal structures. Over 90% of the cells were strongly CD3+, CD4+, and CD8-. There were few clusters of CD20 expressing lymphocytes ( $<5\%$ ) and scattered CD68+ macrophages. Skin samples tested negative for all four primate T lymphotropic virus types including HTLV-I, HTLV-II and all known types of STLV, such as STVL-I, STVL-PH969, STLV-pp1664, and STLV-III by PCR.

**Conclusions:** We report the first case of cutaneous epitheliotropic lymphoma in a baboon and in non-human primates. The immunological phenotype of the neoplastic cells in this baboon is similar to those observed in humans (CD+, CD4+, CD8-) which is different from those observed in dogs (CD3+CD4-, CD8+).

#### **SP-77: THE TRANSCRIPTIONAL RESPONSE OF ENDOTHELIAL CELLS TO STATIN TREATMENT IN VIVO**

Katherine Turnbull, Audrey Cleuren, David Ginsburg

Statins inhibit hydroxymethylglutaryl-CoA reductase, an enzyme in the cholesterol synthesis pathway, and are widely prescribed for cardiovascular risk reduction. Besides lowering cholesterol, statins have been proposed to further reduce cardiovascular risk via other pleiotropic anti-inflammatory and pro-angiogenic effects, potentially mediated through the endothelium. To study the effects of statin treatment on the endothelium, 3 male mice were treated orally with atorvastatin daily for one week, and 3 untreated males were used as controls. To characterize changes in the endothelial cell (EC) transcriptome induced by statin treatment, we applied the Translating Ribosome Affinity Purification (TRAP) technique, tagging the ribosomal protein RPL22 with hemagglutinin (HA) in all ECs in the mouse *in vivo* by crossing a conditional Rpl22-HA allele with a Tek-Cre recombinase transgene. After cycloheximide perfusion to freeze polysome:mRNA complexes, tissues (brain, heart, kidney, and liver) were harvested. EC-specific mRNA was isolated through immunoprecipitation and subjected to high throughput RNA sequencing analysis. In addition to the identification of pan-endothelial and vascular bed-specific EC transcripts, unsupervised hierarchical clustering of EC genes indicated clear (tissue-specific) effects of statin treatment. Sixty-six genes were affected by atorvastatin in ECs of all tissues, of which several were validated using *in situ* hybridization assays. In conclusion, we are able to isolate endothelial-specific mRNA *in vivo* and observed distinct heterogeneity between expression profiles of different vascular beds, as well as in response to treatment with atorvastatin.

#### **SP-78: BACKGROUND LESIONS IN AFRICAN GREEN MONKEYS (CHLOROCEBUS AETHIOPS SABAEUS)**

Victoria Savinon, Pompei Bolfa, Amy Beierschmitt

African green monkeys (*Chlorocebus aethiops sabaeus*) were introduced to the West Indies during the 17<sup>th</sup> century, and are considered to be a “pest species” in St. Kitts. There is a paucity on background lesions or non-lesions in these known models for aging diseases, type 2 diabetes, hypertension, neurobiology and behavior, traumatic injury or toxicology. We describe histological findings of 21 different organs from 73 African greens (48 males and 25 females, 80.82% adults and 19.18% juveniles) from a mixture of colony born and wild caught, used in different studies. All organs examined had histological changes, with the most commonly affected being the lungs (78.08%), kidney (73.97%), liver (68.49%), spleen (68.49%), followed by tongue, skeletal muscle, lymph node, adrenal gland, heart, salivary gland, pancreas, uterus, and testicle. The most common lesion seen in many organs was focal lymphoplasmacytic infiltration. The main changes in the lungs were compatible with silicate pneumoconiosis (68.49%), which was also seen in other species in St. Kitts. Two monkeys had renal intratubular

calcium oxalate monohydrate crystals. Two cases of pancreatic islet hyperplasia and pancreatic lipomatosis were seen. Cystacanths of *Oncicola venezuelensis* (Acanthocephala: Oligacanthorhynchiadae) were found in the tunica vaginalis of a male monkey showing that they can be paratenic hosts. Pregnancy associated vascular remodeling was seen in multiparous females. There was a high prevalence of *Sarcocystis* spp. intramuscular cysts seen in 34 (46.58%) monkeys. Background lesions in African green monkeys are common and diverse involving all major organs examined in this study.

#### **SP-79: DYSREGULATION OF THE MICRORNAS LET-7A AND LET-7B IN CANINE UROTHELIAL CARCINOMA**

Caitlyn Ridenour, Nelly Elshafie, Pierre Deshuillers, Deborah Knapp, Jose Ramos-Vara, Andrea Santos

MicroRNAs (miRs) are molecular regulators of cell development and fate; their dysregulation can lead to carcinogenesis. Studies in human urothelial carcinomas (UC) have shown altered expression of miRs, which can be used as biomarkers for diagnostic testing, prognostic classification, disease monitoring, and as therapeutic targets. However, few studies in dogs are available. UC are the most common bladder tumors in dogs; deciphering miRs' role in canine UC would provide new tools for biomarker discovery and insights into cancer biology. The study's goals were to confirm that amplifiable miRs can be isolated from formalin-fixed, paraffin-embedded (FFPE) bladder tissue and compare the expression of miRs in non-cancerous and UC specimens. Tissue cores were acquired by selecting areas on H&E-stained tissue sections and punching out corresponding areas in FFPE tissue blocks using a biopsy needle. MiRs were isolated using miRNeasy FFPE kit (QIAGEN, CA). RNU6B, mir-21, 103, 155, let-7a and let-7b were quantified by RT-qPCR using the miScript PCR system (QIAGEN) on a QuantStudio3 thermocycler (ThermoFisher Scientific, MA). Relative expression was calculated by the  $2^{-\Delta\Delta C_t}$  method with RNU6B as the normalizer, and compared by *t*-test. Let-7a is downregulated while let-7b is upregulated in canine UC tissue, as in human UC. The let-7 family is involved in cell cycle and tumor suppression. No expression changes were detected for miR-21, 103, and 155. This study shows that miRs can be isolated from FFPE bladder tissue allowing miR expression studies in archived samples. Moreover, two dysregulated miRs were identified, to be used for future biomarker research.

#### **SP-80: DETECTION OF HEPATOZOON AMERICANUM IN GULF COAST TICKS FROM OKTIBBEHA COUNTY, MS**

Alexandra Frankovich, Andrea Varela-Stokes, John Stokes, Shaira Rivera

American Canine Hepatozoonosis (ACH) is a protozoal disease caused by *Hepatozoon americanum*. This organism is transmitted to canines when they ingest the definitive host, the Gulf Coast tick (*Amblyomma maculatum*; GCT) or a paratenic host (rodents and rabbits). There is currently no treatment for eliminating protozoa in the infected canine. The main source of infection, tick or paratenic host, has not yet been identified for ACH making it difficult to develop preventative measures. Data on disease prevalence and distribution are reliant on detection in the diseased canine. Currently,

there is no data detailing *H. americanum* prevalence in the tick vector. This study aimed to fill the gap in knowledge by investigating GCT infection prevalence. We collected 129 adult GCTs from 3 different sites in Oktibbeha Co., MS and extracted DNA from half or whole ticks. We used a TaqMan quantitative PCR assay to test for *H. americanum*. No half tick extracts were positive using conservative threshold levels; two whole tick extracts had low copy numbers but could not be confirmed. Thus, prevalence of *H. americanum* in these GCTs was 0% which supports the null hypothesis. Thus far, results suggest GCTs in this area may not be a source of *H. americanum* infection. Future studies targeting areas with ACH cases and investigating infection rates of nymphal GCTs and various paratenic hosts will offer more insight on the main transmission route for ACH here. This information could be crucial for the development of improved methods for prevention.

#### **SP-81: NASAL ADENOCARCINOMA IN A CAT**

Clinson Lui, Jasmine Choi, Tracey McNamara, Ana Alcaraz

A 10-year-old stray female domestic medium hair cat presented to the emergency room with respiratory difficulties, dry bloody discharge from the right nostril and a mild swelling on the right bridge of the nose. The cat was depressed and underweight (BCS 2/5) with an estimated 8% dehydration. On auscultation, there were crackles on inspiration with dyspnea and mucopurulent-to-serosanguineous discharge from the nose. There was partial distortion of the face by a mass on the right side. Intranasal samples taken for cytology showed that the animal had nasal carcinoma. Over the next few days, the animal had a rapid decline in health. The patient was euthanized 9 days after initial presentation. On necropsy, a 2 x 3 cm mass was present occupying both nasal cavities up to the ethmoid turbinate in the left nasal cavity. The mass occluded both sides of the nasal cavity completely, invading and deviating the nasal septum. The mass was locally invasive, eroding bone of the nose bridge. The hard palate and the left ventral venous plexus were also compressed ventrally by the mass. Nasal tumors in cats are not particularly common but almost all nasal tumors are malignant. Histopathology of the mass confirmed the cytology diagnosis of adenocarcinoma.

#### **SP-82: HEMOLYTIC ANEMIA, SPHEROCYTOSIS, AND THROMBOCYTOPENIA ASSOCIATED WITH HONEY BEE ENVENOMATION IN A DOG: IMMUNE MEDIATED OR NOT?**

Rajeev Nair, Mary Anna Thrall, Emily Riddle

A 14 month-old male Belgian breed dog from St. Kitts, West Indies, presented for massive honey bee envenomation. Hemolytic anemia, echinocytosis, spherocytosis, thrombocytopenia, hemoglobinemia and hemoglobinuria developed following envenomation. The dog was treated supportively and with glucocorticosteroids and recovered. Spherocytosis and hemolysis in patients with massive bee envenomation may be immune-mediated, but are more likely due to the direct toxic effects of the primary components of bee venom, melittin and phospholipase A<sub>2</sub> (PLA<sub>2</sub>). Melittin causes hemolysis by causing large pores to form in erythrocytes resulting in leakage of hemoglobin, and it also causes spectrin stiffening and resultant echinocyte and spherocyte formation. Melittin also stimulates PLA<sub>2</sub>, a hydrolase that causes

echinocytosis and spherocytosis in vivo and in vitro. Thrombocytopenia is also likely a result of the direct cytotoxic effects PLA<sub>2</sub> and melittin, both of which induce mitochondrial breakdown resulting in apoptosis. Alternatively, an immune mediated component of erythrocyte and platelet destruction may be present because of autoantibody formation against damaged erythrocyte and platelet membranes.

#### **SP-83: URINARY BLADDER BOTRYOID RHABDOMYOSARCOMA WITH EXTENSIVE METASTASIS IN AN 18-MONTH-OLD ROTTWEILER**

Ashleigh Shoemaker, Leah Stein, Jason Couto, Dodd Sledge, Matti Kiupel

An 18-month-old, intact female Rottweiler had a 6-month history of bloody vulvar discharge and progressive weight loss, lethargy, and coughing. Ultrasonography revealed a large mass in the caudal abdomen and multiple masses in the thorax. The dog was euthanized due to her deteriorating condition. On gross post-mortem examination, the urinary bladder contained a large, multinodular mucosal mass originating from the trigone. The caudal abdominal lymph nodes were enlarged, and the right kidney contained a well circumscribed nodule. The mediastinum, pericardial sac, costal pleura, and pulmonary pleura were covered by hundreds of variably sized, irregularly shaped, centrally umbilicated nodules. Similar nodules largely replaced the caudal lung lobes. Histologically, the urinary bladder mass consisted of a proliferation of highly anaplastic cells arranged in streams and sheets that expanded the suburothelial stroma and were distinct from the overlying, histologically normal urothelium. Nodular proliferations of similar cells were scattered throughout the lungs, right kidney, adrenal gland, and mediastinum. Neoplastic cells were not immunoreactive for CD18 or CK7, but had strong immunoreactivity for desmin. These immunohistochemical results, along with the signalment of the dog, the location of the primary mass, and the histomorphology of the neoplastic cells confirmed this neoplasm as a botryoid rhabdomyosarcoma. Urinary bladder botryoid rhabdomyosarcomas are very rare, but most frequently occur in young, female, large breed dogs that are less than 2 years of age. Metastasis of such tumors is considered exceedingly rare, but the few reported cases parallel the gross appearance and pattern of metastases observed in this case.

#### **SP-84: INTRAGASTRIC INFUSION OF THE VACUOLATING CYTOTOXIN OF *HELICOBACTER PYLORI***

Robin Holland, Kristopher Bosi, Steven Blanke

**Background:** In order to establish an infection and subvert host defenses, pathogenic microorganisms secrete effector molecules that directly interact with host tissues and subsequently disrupt host physiology. Although the consequences of infection are relatively well studied, less is known regarding the specific role that an individual secreted effector plays in pathogenesis. The *Helicobacter pylori* vacuolating cytotoxin (VacA) is a secreted effector that is internalized with host cells and induces mitochondrial dysfunction for reasons that are poorly understood, often due to limitations in evaluating the specific effects of VacA *in vivo*.

**Objective:** Our objective was to evaluate the *in vivo* effects of VacA by exposing gastric tissue to purified VacA through a modality which reciprocates chronic infection with *H. pylori*.

**Methods:** We developed and implemented an intragastric infusion model, whereby intragastric catheters were surgically implanted into the stomachs of mice, and purified VacA was continually infused into the stomach. Tissues were evaluated for histopathology, immunohistofluorescence microscopy, and immunoblotting.

**Results:** Intragastric infusion of VacA resulted in no observable esophageal, intestinal, hepatic, or pulmonary pathology. However, intragastric infusion of VacA resulted in a collapse of gastric pit structure, parietal cell vacuolation, and depletion of gastric mucus. Furthermore, VacA infused mice exhibited an increase in immune cells within the spleen, and had detectable IgM and IgG antibodies against VacA.

**Conclusions:** Altogether, these studies support a model that VacA mediated mitochondrial disruption functions to limit gastric mucus and acid secretion, thereby facilitating the ability of *H. pylori* to establish chronic, lifelong infections with the stomach.

#### **SP-85: MULTIPLE NEUROENDOCRINE NEOPLASIA IN A BULLDOG**

Chelsea Bond, Katie Boes, Kevin Lahmers, Geoffrey Saunders, Nikolaos Dervisis, Jeffrey Ruth

A 12-year-old castrated male bulldog was presented for an incidental grade II/VI heart murmur and abdominal mass identified by a referring veterinarian. Physical examination and hematologic findings were otherwise unremarkable. Computed tomography revealed a 7.4 cm x 8.7 cm x 4.9 cm heart base mass displacing the left cranial lung lobe, a 10 cm x 4.9 cm x 4.8 cm ileocolic mass invading the portal vein, enlarged mediastinal and mesenteric lymph nodes, and hepatosplenic nodules. Fine-needle aspiration of the heart base mass revealed cell-free nuclei and individualized or adherent neoplastic round to polygonal cells with marked anisokaryosis and fine pink cytoplasmic granules consistent with neuroendocrine carcinoma. Core-needle biopsy of the ileocolic mass revealed neoplastic round to polygonal cells subdivided into small packets by fine fibrovascular stroma compatible with neuroendocrine carcinoma. Based on the masses' locations and similar sizes, a diagnosis of multiple neuroendocrine neoplasia was favored. Since surgical excision of the masses had a high mortality risk, and the dog was subclinical for disease, the dog was discharged without further treatment. Of the common heart base tumors in dogs, chemodectoma (paraganglionoma) is the only neoplasm with the observed cytologic findings. Chemodectomas are tumors arising from chemoreceptor organs that are concentrated around major blood vessels, such as the aorta and carotid arteries. These tumors are most frequent in male brachiocephalic dogs, and affected dogs have an increased risk for multiple paragangliomas. This case highlights the importance of recognizing breed predispositions and known tumor behavior and using multimodal diagnostics in disease diagnosis.

### **SP-86: UNUSUAL PRESENTATION AND URINARY TRACT OBSTRUCTION DUE TO DISSEMINATED INTRA-ABDOMINAL EUMYCETOMAS CAUSED BY CURVULARIA SPECIES IN A DOG**

Deborah Chong, Joe Herbert, Derek Spielman, Mark Krockenberger

A 9-year-old female spayed Labrador dog presented with chronic recurrent urinary tract infections and incontinence. On physical examination, 2 large palpable cranial and caudal abdominal masses with a discharging sinus at the ventral midline were noted. Cytology and culture of fine needle aspirate of the caudal mass revealed fungal elements. Surgical exploration revealed the masses to be two of several firm masses, most of which were intraabdominal. These varied in size, adhered to several abdominal organs including the dorsolateral caudal aspect of the bladder, and had an associated clear gelatinous exudate containing black granules. Histopathology confirmed them to be eumycetomas containing numerous irregular brown-pigmented occasionally septate fungal hyphae with some degree of branching. There was a history of a similar intra-abdominal eumycetoma previously removed 3 years ago. Euthanasia was elected following a refractory response to intravenous and oral itraconazole. Necropsy and histopathological examination revealed fungal invasion limited to the serosa of various abdominal organs. A severe unilateral hydronephrosis with tubular dilation of the contralateral kidney was also present, suspected to be due to urinary tract obstruction from compression of the bladder by its associated mass. Amplification and sequence analysis of internal transcribed spacers and the partial large subunit of the 25-28S ribosomal RNA regions of fungus cultured from samples identified it to be *Curvularia* species. These opportunistic soil pathogens are known to cause invasive phaeohyphomycosis usually restricted to subcutaneous lesions, presumably associated with penetrating trauma. The extensive intraabdominal invasion and associated urinary tract obstruction makes this a novel case.

### **SP-87: SUBRETINAL PROTOTHECOSIS IN A DOG**

Christina Dover, Andrew Enders, Jessica Villm, Sarah Barrett

A two-year-old, male castrated Labradoodle presented to the Virginia-Maryland Veterinary Teaching Hospital Emergency Service for retinal detachment in the left eye. Previous history included diabetes insipidus, toxoplasmosis, Lyme disease, ventriculitis, meningitis, and congenital hydrocephalus. Total bullous retinal detachment of the left eye and hyporeflective retinal lesions of the right eye were discovered on physical exam and confirmed by an ophthalmologist. Further diagnostics included complete blood count (CBC), serum chemistry profile, and urinalysis. Results included a mild neutrophilia and monocytosis; mild increases in electrolytes, liver enzymes, cholesterol, triglycerides, and anion gap; and hyposthenuria, consistent with the previous diagnoses and glucocorticosteroid use. Cytology of the subretinal fluid revealed abundant algae compatible with *Prototheca* spp. together with moderate neutrophilic inflammation. The algae were round to ovoid to reniform in shape, measuring 4-16µm in diameter, with an ovoid central nucleus frequently obscured by basophilic granules, and a thin cell wall. Empty casings and melanin pigment were occasionally observed. *Prototheca* are achlorophyllous, unicellular, saprophytic algae that are occasionally pathogenic. The pathogenic species in dogs include *Prototheca wickerhamii*, which causes cutaneous

disease and *Prototheca zopfii*, which causes disseminated disease. Cutaneous forms are found more often in cats, while the disseminated form in dogs can present with varying clinical signs, including ocular, gastrointestinal, and renal symptoms. It was suspected that a debilitated immune system played a role in the pathogenesis of this disease, and the prognosis was grave. Treatment with amphotericin B and itraconazole was instituted, but due to poor quality of life, humane euthanasia was elected.

#### **SP-88: PRIONS ARE COMMONLY PRESENT IN MUSCLE OF CWD-INFECTED ANIMALS**

David Bissinger, Edward Hoover, Candace Mathiason, Clare Hoover, Davin Henderson, Amy Nalls, Nathaniel Denkers

Chronic wasting disease (CWD), a transmissible spongiform encephalopathy occurring naturally in wild cervids, represents a direct and growing threat to wildlife and an as-yet unknown threat to humans and domestic species. Muscle tissue is likely the most significant source of human and domestic animal exposure through the consumption of CWD-contaminated venison. Previous investigations have established the presence of PrP<sup>CWD</sup>, the CWD infectious agent, in muscle harvested from CWD-positive animals, however, its prevalence and distribution in diseased cervids remains unknown. Here we have combined two powerful *in vitro* amyloid amplification techniques, protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC), to detect PrP<sup>CWD</sup> in muscle from experimentally-infected white-tailed deer (*Odocoileus virginianus*). We detected CWD muscle seeding activity in 19 of 25 deer - a prevalence of 76%. Further analysis indicated that 0.1mg of terminal muscle contains the amount of PrP<sup>CWD</sup> equivalent to 10pg of CWD-positive brain – a concentration too low for reliable detection by conventional assays (e.g. immunohistochemistry or western blotting). Analysis of multiple muscle locations demonstrated that CWD prion seeding activity can be detected throughout skeletal muscle sites. These results are important in demonstrating that CWD prions are widely distributed in muscle at terminal. This ultra-sensitive amplification method can be used to track tissue pathways of prion spread during infection.

#### **SP-89: M-PROTEIN DISTRIBUTION AND SELECT POPULATION CHARACTERISTICS IN CANINE PARAPROTEINEMIA**

Christina Jeffries, Mary Leissing, Adam Harris, A Russel Moore

**Background:** Published literature on canine paraproteinemia suggests that IgG and IgA monoclonal gammopathies occur at approximately equal proportion and that biclonal gammopathies are not commonly found. This data comes from studies which often lack a large enough population to confidently draw conclusions.

**Objective:** Determine the distribution of paraproteins, and describe select population characteristics (breed, gender, and neuter status), among dogs with paraproteinemia from a retrospective case set.

**Methods:** The CSU Clinical Pathology Laboratory database was searched for the initial evaluation of cases of paraproteinemia with both serum protein electrophoresis (SPE)

and Immunofixation electrophoresis (IFE) performed between January 2014 and December 2017. Diagnosis, including classification as monoclonal or biclonal and IgG, IgA, or IgM gammopathies was confirmed by a single pathologist. Equivocal results were excluded. Population data was compiled from case histories.

**Results:** Of the 43 cases which met inclusion criteria, 62.79% were IgA (27/43), 23.26% IgG (10/43), and 13.95% IgM (6/43). 58.14% of all gammopathies were considered monoclonal, and the remaining 41.86% were biclonal. IgA gammopathies most commonly appeared biclonal in nature (59.25%, 16/27). IgG and IgM were predominantly monoclonal (90% and 83.3%, respectively). 42.5% of cases were mixed or unknown breed. Golden retrievers were the most frequently represented breed at 15% (6/43). There were 17 (39.5%, 2 intact/15 spayed) females, 24 (55.8%, 3 intact/21 castrated) males and 2 individuals of unknown sex.

**Conclusions:** IgA gammopathies predominated and biclonal appearance was more common than the literature suggests. Additional studies are needed to evaluate the correlation between paraprotein type and diagnosis/prognosis.

#### **SP-90: NECROTIZING FASCIITIS WITH CUTANEOUS REACTIVE HISTOCYTOSIS IN A DOG**

Samantha Hughes, Sharon Yang, Timothy Morgan

**Case Report:** A 2-year-old male neutered Catahoula mix breed dog presented to the Mississippi State University Animal Health Center for acute onset of pain in the forelimbs, dull mentation and inappetence. The dog also had a 1-2-month history of multifocal alopecic nodular skin lesions on the dorsum and flank. Physical exam revealed marked ventral and forelimb edema, and the skin lesions had purulent exudate. The dog was humanely euthanized. On necropsy examination, there was a large amount of red-tinged thick opaque fluid dissecting among the fascial planes of the abdominal wall musculature along with marked edema and hemorrhage. The left axillary lymph node was enlarged containing cavities filled with purulent material. Necrotizing fasciitis and lymphadenitis were confirmed on histopathology, and the exudate cultured *Streptococcus canis*. The chronic skin lesions were consistent with cutaneous reactive histiocytosis, with multifocal epidermal ulcerations.

**Discussion:** Necrotizing fasciitis is a rare condition described in dogs and is often preceded by a *Streptococcus* infection, most commonly *S. canis*, such as this case. The bacteria colonize a minor wound which then invades into the deeper tissues. Within 24 to 48-hours, the infection spreads rapidly causing widespread tissue necrosis with exotoxin and cytokine release causing a rapid decline in the animal. The dog had areas of skin ulceration secondary to the cutaneous reactive histiocytosis, which we propose may have enabled *S. canis* to colonize leading to the necrotizing fasciitis.

## **ACVP Late Breaking Poster Session**

### **LB-01: DISSIMILAR CARCINOMAS IN KIDNEY, BILE DUCT, AND THYROID GLAND OF A CAT**

Stacey Piotrowski, Margaret Miller, Breann Sommer, Deidre Dusold, Jose Ramos-Vara

**Background:** A 16-year-old spayed domestic longhaired cat with a history of lethargy, weight loss, anorexia, left renomegaly, cholelithiasis, and peritoneal effusion was euthanized. At autopsy, masses were found in the left kidney, common bile duct, and left thyroid lobe.

**Objective:** Pathology of multi-organ carcinomas in a cat

**Methods:** Formalin-fixed, paraffin-embedded tissues were evaluated histologically and with immunohistochemistry (IHC) for Pax-8, cytokeratin 7 (CK7), thyroid transcription factor-1 (TTF-1), thyroglobulin, pan-cytokeratins (AE1/AE3), vimentin, CD10, and uroplakin-2.

**Results:** The renal mass involved the cortex and consisted of tubules and solid nests of polyhedral cells with squamous differentiation and keratinization. The neoplastic cells were uniformly positive for AE1/AE3 and variably positive for vimentin, but negative for other markers including uroplakin-2. The common bile duct was dilated and tortuous proximal to the carcinoma, which consisted of tubules and nests of epithelial cells in scirrhous stroma. Neoplastic tissue invaded nearby pancreatic tissue. All neoplastic cells expressed AE1/AE3; 50% expressed CK7; 75%, vimentin. The thyroid mass consisted of compact epithelial cells that invaded the capsule and vasculature. The neoplastic cells expressed Pax-8, TTF-1, and thyroglobulin.

**Conclusions:** Final diagnoses for the three tumors were renal squamous cell carcinoma, cholangiocellular carcinoma, and compact thyroid carcinoma. All were considered primary neoplasms. Squamous cell carcinoma is rare in the kidney and usually derived from pelvic urothelium, but the tumor in this cat lacked continuity with the pelvis and did not express uroplakin-2. Cholangiocellular carcinoma is more commonly intrahepatic in cats. Feline thyroid carcinoma is less common than follicular hyperplasia or adenoma.

### **LB-02: INVESTIGATING THE VALUE OF URINE VOLUME, CREATININE AND CYSTATIN C FOR URINARY BIOMARKERS NORMALIZATION IN NONCLINICAL STUDIES.**

Adeyemi Adedeji, Tony Pourmohamad, Yafei Chen, Jennifer Burkey, Laura Song, Manisha Sonee, James McDuffie

**Background:** Due to urine volume variability, novel urinary biomarkers are often normalized with either creatinine or urine volume. However, given the non-glomerular filtration rate factors that can affect creatinine concentrations, coupled with the obvious variations associated with urine volume, creatinine and urine volume variability may lead to under- or over-estimating the renal biomarkers' concentration following normalization. Cystatin C is thought to have a constant serum concentration, and is

currently being considered as a creatinine replacement for glomerular filtration rate estimation and consequently renal biomarkers' normalization.

**Objective:** The purpose of this study was to see whether the degree of variability associated with creatinine and cystatin c in humans, would translate to preclinical animal species within a nonclinical environment; and compare the variability of urine volume, creatinine and cystatin c, and decide which of these endpoints would be better for renal biomarker normalization.

**Methods:** We compared the variability of cystatin c, creatinine, and urine volume, by using data obtained from a Predictive Safety Testing Consortium database that contained renal biomarker studies performed in rats by all member pharmaceutical companies.

**Results:** We showed that, over time, urine creatinine was less variable than urine cystatin c and urine volume. Renal biomarkers normalized with creatinine, also showed lesser variability relative to normalization with cystatin c and urine volume.

**Conclusion:** To our knowledge, this is the first report that compared the variability of urine volume, cystatin c and creatinine in the context of renal biomarkers' normalization, with creatinine being the best normalization factor in this study.

### **LB-03: IN OVO STUDY OF BLUETONGUE VIRUS: PATHOGENESIS AND THE PATHOGENICITY OF INFECTIOUS BLOOD AND CELL CULTURE PASSAGED VIRUS**

Fabian Lean, Joanne Devlin, David Williams, John Bingham

**Background:** Bluetongue virus (BTV) (genus *Orbivirus*, family *Reoviridae*) is a midge-borne virus that causes vascular pathology in ruminants. In the laboratory, embryonated chicken egg (ECE) is the most susceptible system to BTV infection.

**Objective:** The pathogenesis of BTV and influence of cell culture passage on pathogenicity were investigated using the ECE.

**Methods:** A time course study was conducted using a sheep-derived BTV serotype 3 (BTV-3) South African reference strain. The influence of virus passage history on the pathogenicity *in ovo* was also investigated using insect cells (*Culicoides sonorensis*) and mammalian cells (BSR variant Baby Hamster Kidney cells) passaged BTV-3. The levels of viral antigens were assessed using immunohistochemistry (IHC) against viral non-structural proteins NS1, NS2, NS3/3a and structural protein VP7.

**Results:** The BTV-3 inoculum consisting of sheep blood from clinically-affected sheep produced comparable levels of viral antigens NS1, NS2, NS3/3a and VP7 in the ECE. Viral antigens were observed in the vasculature of the lung, heart, liver, spleen, and chorioallantoic membrane, with no apparent discrimination between the macro and microvasculature. BTV also replicated in neuronal cells within the central nervous

system. Passaging BTV through cell lines attenuated the pathogenicity of the virus, evident by longer survival time and lower levels of viral protein expression *in ovo*.

**Conclusion:** Bluetongue virus exhibited vascular and neuro-tropism in the ECE model. The pathogenicity of BTV was attenuated with cell culture passage. The results of this work have potential implications for large animal pathogenesis studies using BTV-3.

**LB-04: APPARENT LACK OF DRUG ABSORPTION OF BUPRENORPHINE SR™ LAB FORMULATION ADMINISTERED SUBCUTANEOUSLY TO NUDE RATS (RATTUS NORVEGICUS)**

C. Page, Rachel Estrada, R. Hoffman, Travis Mays, Naomi Gades

Forty- five female nude rats, 6 weeks of age, were utilized in an approved Institutional Animal Care and Use Committee protocol to investigate mechanisms and potential treatments associated with brain, spine, and spinal cord metastasis. The analgesic plan included the use of Buprenorphine SR™ LAB (0.6 mg/kg; 0.11 ml/rat) subcutaneously and an oral NSAID delivered via the water. At approximately 3 months post-xenotransplantation, animals were sacrificed. Grossly, nodules were seen in 37 of the rats; these were identified as small, cystic structures, approximately 0.25 cm in diameter, in the subcutis over the shoulders. The cysts and haired skin were submitted for liquid chromatography-tandem mass spectrometry (LC-MS/MS) and histopathology to Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX. Histologically, fibrous connective tissue was mildly infiltrated by macrophages, lymphocytes, and plasma cells. Adjacent blood vessels were rimmed by a mild infiltrate of lymphocytes and plasma cells. The cysts contained variable accumulations of a light pink, proteinaceous fluid. The cause for the cysts could not be determined histologically; there was no evidence of neoplasia. LC-MS/MS analysis revealed the cysts contained buprenorphine. We hypothesize that the lack of T-cells and a cell-mediated immune response in these rats prevented the dissolution of the vehicle and absorption of the buprenorphine. ZooPharm™ does provide a cautionary statement in the information sheet regarding the use of this formulation in nude mice due to skin reactions. However, to our knowledge this is the first report of an apparent lack of absorption of the drug in an immunodeficient animal.

**LB-05: NOVEL PRECLINICAL ORTHOTOPIC CANINE PROSTATE CANCER MODEL FOR DRUG DEVELOPMENT TARGETING THE GASTRIN-RELEASING PEPTIDE RECEPTOR**

Thomas Rosol, Said Elshafae, Li Gong, Shankaran Kothandaraman, Michael Knopp, Michael Tweedle

**Background:** Theranostic drugs under development for prostate cancer (PCa) must be tested in a large animal model prior to human clinical trials.

**Objective:** We developed an orthotopic PCa model in dogs to target the gastrin-releasing peptide receptor family (GRPR) by unique bombesin analogues. Prostate cancer in men overexpress the GRPR compared to hyperplastic tissue.

**Methods:** Canine Ace-1 PCa cells were stably expressed with the homologous human or canine GRPr. The cells were validated by radioligand binding of  $^{125}\text{-I}$ -bombesin (BBN). Cells were implanted orthotopically into the prostates of immunosuppressed beagles using ultrasound. Cells were also inoculated subcutaneously. At 4-6 weeks, the lateral prostatic artery was catheterized (via the carotid artery) and a near infrared fluorescent GRPr ligand (100 nmoles 800-G-Abz4-t-BBN) was injected. 24-48 h post catheterization, euthanasia and necropsy were performed. A fluobeam imager was used to detect the tumor fluorescence.

**Results:** Ligand binding was equivalent to human and canine GRPr. Ace-1-k9GRPr strongly expressed GRPr compared to human PC-3 cells and were less immunogenic than Ace-1-huGRPr cells in vivo. Ace-1-k9GRPr cells grew considerably faster outside of the prostate gland. Fluorescent imaging revealed multifocal tumor foci inside the prostate that were confirmed by histopathology. Ace-1-k9GRPr cells formed tumors with cribriform patterns and central necrosis. There were occasional extraprostatic and lymph node metastases. Subcutaneously, Ace-1-k9GRPr cells induced desmoplasia and invaded fat and muscle.

**Conclusions:** Ace-1-k9GRPr tumors maintained the expression of GRPr in vivo and will be useful to test the safety and efficacy of radioactive bombesin peptides in a large animal model of PCa.

#### **LB-06: EFFECT OF ANTI-COAGULANT AND MAGNESIUM CHLORIDE ON FIBRINOGEN MEASUREMENT**

Natalie Courtman, Brett Tennent-Brown, Crystal Leung, Emily Driscoll

**Aims:** To assess the agreement between fibrinogen measurements in EDTA and citrate plasma samples, and to explore the effect of  $\text{MgCl}_2$ .

**Method:** Fibrinogen concentration was measured by modified Clauss method with an automated coagulation analyser (STA-Liquid Fib assay, STA Compact, STAGO) on EDTA and citrate plasma samples from 74 healthy horses and 7 sick horses with an increased fibrinogen concentration. Measurement of fibrinogen in EDTA and citrate plasma samples from 20 of the healthy horses and the 7 sick horses was then repeated with the addition of  $\text{MgCl}_2$  to the reagent buffer.

**Results:** Comparison of EDTA versus citrate plasma fibrinogen measurements identified a constant negative bias in healthy horses, and a negative proportional negative bias in horses with increased fibrinogen concentration. Addition of  $\text{MgCl}_2$  increased measured fibrinogen concentration in EDTA plasma samples but had no effect on citrate plasma samples. For EDTA samples, the magnitude of difference before and after  $\text{MgCl}_2$  addition was positively correlated with mean fibrinogen concentration.

**Conclusion:** Addition of  $\text{MgCl}_2$  to EDTA plasma samples increases the measured fibrinogen concentration in horses but has no effect on citrate plasma samples. Further

work is required to determine the optimal magnesium supplementation for measurement of fibrinogen on EDTA plasma by the modified Clauss method.

#### **LB-07: APPLES TO APPLES: AIRWAY CALIBER VERSUS AIRWAY WALL CONSTITUENTS**

David Meyerholz, Amanda Beck

Scoring of lung tissues often involves specific evaluation of airways walls and if there is increased or decreased collagen and/or smooth muscle. Alterations of these tissue parameters may be markers of diseases like asthma, chronic obstructive pulmonary disease or cystic fibrosis. The mouse lung has airways that exhibit monopodial branching and intrapulmonary airways are exclusively bronchioles (i.e. no intra-pulmonary bronchi). As such, many airways can look alike for analysis. We tested if the airway caliber in wildtype mice could influence the detection and scoring of airway collagen or smooth muscle composition. We examined 15 mice from archival studies that had appropriate institutional approvals. Airway lumen diameter as well as wall thickness (sum of epithelium, smooth muscle and collagen) measurements of the 2 largest and 2 smallest airways were taken. These were then cumulatively analyzed for wall thickness (y axis) and airway lumen diameter (x axis). There was a significant positive slope (slope = 0.046 and  $P=0.0017$ ). We then grouped the data into large (lumen >130 microns) and small (lumen <130 microns) airways and compared the wall thickness, which was significantly thicker in the larger airways  $P<0.0001$ . These data indicate that measurement of airways requires analysis of similar sized airways for accurate assessment and to minimize normal variations in thickness associated with airway caliber.

#### **LB-08: RHABDOMYOSARCOMA IN A GECKO**

Ethan Biswell, Jessica Hanlon, Lorraine Corriveau, José Ramos-Vara

The tail from a 9.5-year-old, intact female Leopard Gecko (*Eublepharis macularius*) with a 2 cm mass on the craniodorsal aspect was submitted for histopathology after surgical amputation. Grossly, this mass was firm, and pale tan on cut section. Histologically, the dermis and deeper soft tissues were replaced by an unencapsulated neoplastic proliferation of intersecting bundles of spindle cells with variable amounts of eosinophilic cytoplasm with rare faint cross-striations and occasionally with a large intracytoplasmic clear vacuole, surrounded by variable amounts of fine fibrovascular stroma. Nuclear pleomorphism was marked with a wide range of number, size and shape of nuclei, which typically had vesicular chromatin and at least one prominent nucleolus.

Centralized nuclei were typical but their long axis was commonly not parallel to the cytoplasm's. Strap cells, fewer racket cells, and scattered small and round mononuclear cells were present within this mass. There were 0-2 mitotic figures per 400X field. The deepest portion of this growth merged with striated muscle undergoing degeneration and regeneration. Immunohistochemically, the more differentiated neoplastic cells were positive for actin muscle (AM), actin sarcomeric (ASAR), and Desmin; and negative for MyoD1 and smooth muscle actin. Therefore, the mass was diagnosed as a pleomorphic rhabdomyosarcoma. Rhabdomyosarcomas are overall rare in animals. In a retrospective study consisting of 381 leopard geckos, there were 21 cases of neoplasia

with only 2 being rhabdomyosarcomas (location not mentioned). To the best of the authors' knowledge, this is the first detailed histologic and immunohistochemical study of this tumor in this species.

#### **LB-09: ANGIOMATOID MORPHOLOGIC VARIANT OF CANINE CUTANEOUS PLASMACYTOMA**

Eric Cassmann, Amanda Fales-Williams

**Background:** Canine cutaneous plasmacytomas occur in humans, dogs and cats. A pseudoglandular variant is described in dogs, while in humans an angiomatoid variant is well recognized.

**Case presentation:** A 14-year-old spayed female mixed breed dog presented for excisional surgical biopsy of multiple tumors along the ventral abdomen. Grossly, the tumor was pink, raised and polypoid. Histologically, most of the tumor was consistent with an archetypal cutaneous plasmacytoma. Regionally a portion of the tumor exhibited an atypical growth pattern characterized by round anastomosing angiomatoid channels lined by neoplastic plasma cells filled with red blood cells or eosinophilic material. Adjacent to these structures there was a prominent vascular stroma. The pattern was interpreted as an angiomatoid variant. Neoplastic cells lining the vascular-like spaces were immunopositive for MUM-1. Concurrently neoplastic cells were immunonegative for Factor VIII and CD31.

**Discussion:** This case demonstrates a morphologically distinct variant of canine cutaneous plasmacytoma. This case was similar to and different from the pseudoglandular variant previously reported in dogs. Similarly, there were dilated luminal spaces lined by neoplastic plasma cells filled with red blood cells or eosinophilic material. Divergently, the structures were adjacent to a region of dense stromal vascularization. The angiomatoid pattern in human plasma cell tumors is thought to be a recapitulation of vascular structures due to VEGF stimulation. We diagnosed an angiomatoid plasma cell tumor based on these criteria: the degree of adjacent stromal vascularization, the morphology of luminal spaces, and the presence of intraluminal red blood cells.

#### **LB-10: OVALICIN ALLEVIATED PRURITIC AND INFLAMMATORY SIGNALING THROUGH INHIBITION OF IL-31 AND ROS IN DH82 CELLS**

Yongbaek Kim, Sung-Hyun Hwang

**Background:** Pruritus induced by environmental and food factors has been increased in dogs. Various factors of immune and inflammatory systems play an important role in pruritus and atopic dermatitis patients. Despite of immense side effects, steroids are the choice of drug. Ovalicin is an extract from *Cordyceps militaris* and has been used for dandruff treatment and agricultural insect control. However, the effects of ovalicin on immune system are rarely studied.

**Objective:** This study was performed to examine the effect of ovalicin on the intracellular signaling molecules that involve in the development of pruritus and inflammatory response.

**Methods:** A canine macrophage cell line, DH82, was stimulated by lipopolysaccharide. Following the treatment with ovalicin, the expression levels of signaling molecules were determined by RT-PCR and Western blot assay.

**Results:** Expressions of IL-31, its receptor, and downstream signaling molecules were significantly reduced by ovalicin treatment in stimulated DH82 cells. Furthermore, intracellular calcium was decreased detected in ovalicin treated DH82, which led to the decreases of transient receptor potential vanilloid 1 (TRPV1) and histamine receptor. Ovalicin treatment suppressed the expression of inflammation-related molecules including inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX2) and NF-kB expression in LPS stimulated DH82 cells.

**Conclusions:** Our data indicated that ovalicin could be a potential candidate of therapeutic agent for atopic dermatitis with pruritus in veterinary medicine, which warrants in vivo studies.

#### **LB-11: INFLUENCE OF DECALCIFICATION METHODS FOR ION CHANNEL IMMUNOHISTOCHEMISTRY: A PILOT STUDY**

J. Goeken, David Meyerholz

Immunohistochemical detection of ion channels such as cystic fibrosis transmembrane conductance regulator (CFTR) are important in the study of many diseases. In CF, airways are a common location for CF related disease and these airways sometimes need to be decalcified due to hard or even calcified cartilage. The decalcification procedure can be performed but in some cases the ability to detect CFTR was apparently compromised. We studied pancreas because of the high CFTR expression. The tissues were serially sectioned and fixed (10% neutral buffered formalin, NBF) for ~1 week and then placed into either NBF, formic acid(FA) or EDTA for up to 1 more week (1h to 7 days). IHC for CFTR was performed and then immunostaining analyzed using CellSens software and quantified as a percent of total area and compared using repeated measures ANOVA followed by Tukey's multiple comparison test. In the repeated measures ANOVA, there was a significance ( $P=0.0249$ ) that implied the means for each group were not the same. Group specific comparisons for CFTR immunostaining was not significant for NBF versus EDTA or FA, but FA had significantly reduced immunostaining compared to EDTA, ( $P<0.05$ ). These results suggest the CFTR antigen may be sensitive to acid –based decalcification and that EDTA would be the preferred decalcification method. This test also highlighted the ability of digital pathology to evaluate the effects on immunostaining.

#### **LB-12: CHARACTERIZATION OF DISTINCT MECHANISM OF AGONIST-INDUCED CANINE PLATELET ACTIVATION**

Preeti Chaudhary, Soochong Kim

Platelet activation plays a major role in hemostasis and thrombosis. Various agonists including adenosine diphosphate (ADP) and thrombin interact with G protein-coupled receptors which transduce signals from the cell surface to intracellular effectors through various G proteins. However, this agonists-induced platelet activation in companion animals is poorly understood. This study was designed to characterize the platelet response to various agonists in canine platelets. We found that 2-methylthio-ADP (2-MeSADP)-induced canine platelet aggregation was blocked by either P2Y<sub>1</sub> receptor antagonist MRS2179 or P2Y<sub>12</sub> receptor antagonist AR-C69931MX, suggesting that co-activation of both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptor is required for ADP-induced platelet aggregation. Thrombin-induced canine platelet aggregation was inhibited by either protein kinase C inhibitor GF109203X or AR-C69931MX, suggesting that thrombin requires secreted ADP to induce platelet aggregation in canine platelets. In addition, thrombin-mediated Akt phosphorylation was inhibited by GF109203X or AR-C69931MX, indicating that thrombin causes G<sub>i</sub> stimulation through P2Y<sub>12</sub> receptor by secreted ADP in canine platelets. Unlike human and murine platelets, PAR4-activating peptide AYPGKF failed to cause canine platelet aggregation. Moreover, PAR1-activating peptide SLLRN or co-stimulation of SLLRN and AYPGKF failed to induce canine platelet aggregation, suggesting a different underlying mechanism for thrombin-induced platelet aggregation in dogs compared to human and mouse. We conclude that ADP induces platelet aggregation through P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors in dogs. Thrombin requires secreted ADP to induce platelet aggregation by G<sub>i</sub> stimulation via P2Y<sub>12</sub> receptor activation in canine platelets, and selective stimulation of either PAR1 or PAR4 is not sufficient to cause platelet aggregation in dogs.

### **LB-13: IDENTIFICATION OF A NOVEL SIADENOVIRUS IN A COCKATIEL WITH CHRONIC HEPATOPATHY**

Eric Cassmann, Bianca Zaffarano, Qi Chen, Ganwu Li, Joseph Haynes

**Background:** Adenoviruses affecting avian species are found within three genera: *Atadenovirus*, *Aviadenovirus* and *Siadenovirus*. There are few pathogenic adenoviruses in psittacines, but none of these viruses have been reported in cockatiels (*Nymphicus hollandicus*). Furthermore, siadenovirus infections have only been reported in budgerigars, a plum-headed parakeet and an umbrella cockatoo. This is the first time a siadenovirus has been identified in a cockatiel; the case is presented combining results from histopathology and next generation sequencing.

**Case presentation:** Nine months after a 15-year-old female cockatiel received empirical treatment for unspecified hepatopathy, she re-presented with advanced disease. The patient received palliative treatment but died four days later. At necropsy there was hepatomegaly. The liver was nodular and mottled green-to-brown in color. Fibrous adhesions of the air sac to the liver capsule were present. Microscopically, fibrosis dissected through the parenchyma and incorporated areas of biliary proliferation. The lobular architecture of the liver was distorted. Hepatocytes were occasionally vacuolated and swollen, and there was multifocal individual hepatocyte necrosis. Frequently intranuclear basophilic viral inclusions were present in hepatocytes and renal tubular epithelium. Next generation sequencing of pooled liver/kidney was able to assemble the complete coding sequence of the siadenovirus hexon gene.

**Conclusion:** The hexon gene encodes the major viral capsid protein and is used for phylogenetic analysis of adenoviruses. Phylogenetic analysis demonstrated that the virus exemplar in this case, cockatiel adenovirus 1, is most closely related to budgerigar adenovirus 1, suggesting it is a novel siadenovirus.

#### **LB-14: FIRST CASE OF FATAL EQUINE MENINGOENCEPHALITIS CAUSED BY HALICEPHALOBUS GINGIVALIS IN MEXICO**

Vicente Avila, Zazil Vázquez, Francisco Carvallo, Gerardo Salas, José Ramírez, Ramiro Calderón, Luay Martínez

Infections of the central nervous system (CNS) of horses are some of the most devastating and frequent fatal diseases. Virus and protozoa are probably the most frequently diagnosed causal agents in these infections. Verminous encephalitis is rare in horses; this aberrant nematode larval migration in the CNS is termed cerebrospinal nematodiasis and one of the main etiological agents involved in this illness is *Halicephalobus gingivalis*. This soil nematode has been associated with several fatal meningoencephalitis reports worldwide, however, it had never been diagnosed as causative of disease in horses of Mexico. A 10 year-old Iberian horse was submitted to the Large Animal Hospital of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, with severe ataxia, nystagmus and posterior recumbence. Despite treatment, no improvement of neurological signs was achieved and the horse was subjected to euthanasia, and submitted to postmortem examination. No gross alterations were found at necropsy. Microscopic examination revealed granulomatous meningoencephalitis and vasculitis, mostly in brain stem and cerebellum, as well as intralesional adult nematodes, larvae and eggs, compatible with *H. gingivalis*. Polymerase chain reaction (PCR) for the nuclear large subunit ribosomal RNA gene (LSU rDNA) was performed on formalin-fixed and paraffin wax-embedded sections of brain. A 900 bp was amplified and sequenced. BLAST analysis of attained sequence identified the nematode agent as *H. gingivalis*. To our knowledge, this is the first confirmed report of Halicephalobiasis in Mexico.

#### **LB-15: CONGENITAL ATLANTOAXIAL AND FORAMEN MAGNUM MALFORMATION IN A SLENDER-TAILED MEERKAT**

Michelle Evans, Sushan Han, Eric Klaphake, Matthew Johnston

Four three-month old slender-tailed meerkats (*Suricata suricatta*) were presented to the zoo veterinary staff for routine vaccines and wellness examinations. All of the animals were anesthetized with isoflurane and whole body radiographs were taken. A presumed congenital abnormality was found on radiographs of the smallest meerkat. The malformation showed decreased osseous coverage of the dorsal aspect of the foramen magnum, atlas, and axis. No neurologic signs or other abnormalities were reported before the meerkat was found dead two months later following a severe hail storm. Postmortem CT showed increased size of the foramen magnum and atlantoaxial joints. Necropsy revealed no obvious cause of death or trauma. There was marked laxity in the atlanto-occipital and atlantoaxial joints with dynamic subluxation of the atlantoaxial joint when the head was manipulated. The dorsal aspect of the spinal cord was exposed in both joint spaces, and the caudal aspect of the brainstem was seen through the

foramen magnum. Histology of the spinal cord was not possible due to two freeze-thaw cycles prior to postmortem examination. To our knowledge, this is the first reported case of a foramen magnum and atlantoaxial malformation in this species. While the spinal abnormalities did not lead to clinical disease in this specific animal, congenital deformities of the cervical spine and subsequent atlantoaxial subluxation should be considered as a differential for neurologic signs in meerkats.

#### **LB-16: EQUINE PROTOZOAL ABORTION ASSOCIATED WITH NEOSPOROSIS**

Joseph Anderson, Jitender Dubey, Derron Alves, Donald Marshall

**Background:** A female quarter horse fetus and placenta were submitted for necropsy after being aborted on day 280 of gestation at a farm in Montana. The only reported medical history was the mare's progesterone levels had been low on days 14, 75, and 150, and she had been receiving Altrenogest since day 14.

**Methods:** Fetal lung, heart, liver, skeletal muscle, tongue, placenta, and brain were collected, fixed, and embedded in paraffin for routine histopathologic evaluation. H&E and immunohistochemical (IHC) stains were applied to these sections of tissues. Transmission electron microscopy (TEM) was performed on lung tissue.

**Results:** Histologically, pulmonary alveolar septa were fragmented and necrotic and alveoli were filled with necrotic debris and alveolar macrophages. Alveolar epithelial cells were expanded by multiple protozoal tachyzoites. The placenta was diffusely necrotic while the heart, liver, and skeletal muscle had moderate multifocal lymphohistiocytic inflammation. The heart and liver also contained protozoal tachyzoites within cardiomyocytes and hepatocytes, respectively. IHC using polyclonal *Neospora caninum* polyclonal antibodies was multifocally positive in all submitted tissues. TEM demonstrated numerous tachyzoites with morphology consistent with *Neospora spp.*

**Conclusions:** The histologic, immunohistochemical, and TEM findings are consistent with equine abortion associated with *Neospora spp.* Previous reports indicate a transplacental mode of infection from mare to fetus. Neosporosis is a rarely reported cause of equine abortion, with a previous report being histologically confirmed in only one instance.

#### **LB-17: NEMATODE LARVA MIGRANS IN KIWI (APTERYX SPP.): AN INDIRECT EFFECT OF INVASIVE MAMMALS ON NATIVE BIRDS**

Wendi Roe, Kristene Gedy, Brett Gartrell, Fernanda Castillo-Alcala, Adrienne French

**Background:** *Larva migrans* results from the migration of larval nematodes within the tissues of a host. Multiple cases of visceral and neural *larva migrans* have been identified at necropsy in kiwi (*Apteryx spp.*), an endangered native bird of New Zealand. The histomorphology of the larvae suggests origin from the nematode genus *Toxocara*.

**Objective:** To identify the specific nematode organism or organisms causing *larva migrans* in kiwi.

**Methods:** PCR was run on DNA extracted from archival formalin-fixed, paraffin-embedded lung and/or brain sections from five cases of histologically diagnosed nematode *larva migrans* in kiwi. Several different primer sets were sourced from the literature, targeting the ITS-2 or 18S regions of the nuclear ribosomal DNA of ascarid nematodes. Sanger sequencing was performed on positive PCR products.

**Results:** Four out of the five cases returned positive PCR results to one or more of the primer sets used. The resultant DNA sequences in all four cases aligned most closely with sequences from *Toxocara cati* present in GenBank<sup>®</sup>, with e-values ranging from  $1e^{-55}$  to  $4.48e^{-148}$ . One case was PCR negative to all nematode primer sets tested, although successful extraction of DNA was confirmed by use of kiwi-specific primers.

**Conclusion:** Results implicate *Toxocara cati* as at least one cause for *larva migrans* in kiwi, representing spillover from an introduced mammalian host (the domestic cat) and providing evidence of an indirect effect of the mammalian invasion of New Zealand ecosystems.

#### **LB-18: HISTOLOGY GUIDED MASS SPECTROMETRY PROFILING ACCURATELY DIFFERENTIATES SMALL CELL LYMPHOMA AND INFLAMMATORY BOWEL DISEASE IN CATS**

Matthew Powell, Sina Marsilio, Erin Seeley, Katy Smoot, Shelley Newman, Jan Suchodolski, Jonathan Lidbury, Joerg Steiner

**Background:** Differentiation of small cell lymphoma (SCL) and idiopathic inflammatory bowel disease (IBD) remains challenging in cats with chronic signs of gastrointestinal disease. However, such differentiation is clinically important because the treatment of intestinal lymphoma involves chemotherapeutic agents with significant side effects that could impact the morbidity of the cat.

**Objective:** Our objective was to use histology guided mass spectrometry profiling to generate *in situ* molecular fingerprinting from formalin-fixed paraffin-embedded (FFPE) tissue sections to distinguish IBD from SCL in cats with chronic signs of gastrointestinal disease.

**Methods:** FFPE tissue sections from 44 cats with chronic signs of gastrointestinal disease (25 SCL and 19 IBD) were deparaffinized, underwent antigen retrieval, and were subjected to on-tissue tryptic digestion. Mass spectra were collected from histopathologically preselected areas of monomorphic or pleomorphic lymphocytic populations (50-micron target regions).

**Results:** A linear discriminant analysis classification algorithm was created using the acquired mass spectral data, resulting in 94.3% accuracy when performing a leave-10%-out internal cross validation assessment. A total of 211 peaks were used to build the model, of which, 194 were found to be statistically significant between the two groups after applying a Bonferroni correction.

**Conclusions:** Results indicate that mass spectrometry may be a powerful tool for accurate differentiation of feline SCL and IBD. Further studies are currently in progress to validate these results using an independent cohort of samples.

### **LB-19: ARRHYTHMIC RIGHT VENTRICULAR CARDIOMYOPATHY IN A RHESUS MACAQUE (MACACA MULATTA)**

Patricia Zerfas, Julie Mattison, Meghan Connolly, Michael Eckhaus

Arrhythmic right ventricular cardiomyopathy (ARVC), is a human disease primarily of young athletes, in which gene mutations alter intercalated disc proteins that results in a fibro-fatty replacement, arrhythmias and sudden death. A 19-year-old obese female rhesus macaque was diagnosed with ARVC at necropsy. The macaque presented with subcutaneous edema of the extremities, ascites and pleural effusion. Thoracic radiographs revealed an enlarged rounded heart which occupied the majority of the thoracic cavity. The heart was enlarged asymmetrically with severe dilation of the right ventricle. The right ventricle had significantly reduced visible muscle and was primarily composed of adipose tissue measuring 0.45 cm in thickness. Histological examination of the myocardium revealed severe infiltration of adipose tissue within the wall of the right ventricle with atrophy and loss of myocytes. Remaining myocytes were hypertrophied with karyomegaly. There was minimal to moderate interstitial fibrosis present in areas adjacent to existing myocytes and adipocytes. Focal mild to moderate fibrosis was also noted in the interventricular septum. On transmission electron microscopy abnormalities were seen involving the intercalated discs and increased numbers of peroxisomes with associated lipid production. This is the first case of a non-human primate to be diagnosed with ARVC with similarities to the human condition.

### **LB-20: NASAL PHAEOPHYCOMYCOSIS AND PULMONARY PROTOTHECOSIS IN AN EQUINE**

Yianelly Rodriguez Ruiz, Ebony Gilbreath-McCloud

Nasal phaeophycomycosis and pulmonary protothecosis were diagnosed in a 10-year-old female Tennessee Walking Horse. The patient presented for dyspnea. Radiographic findings included a soft tissue opacity in the left nasal cavity; endoscopy was attempted but the mass obstructed the nasal meatus. Horses are obligate nasal breathers and due to the poor prognosis of an obstructing mass, euthanasia was elected. On necropsy, multiple firm nodules (granulomas) were infiltrating the left nasal conchae and ethmoid turbinates. There were also two focally extensive granulomatous areas in the lung. Histology of the nasal tissue revealed granulomatous inflammation with dematiaceous spherical fungal organisms. Lung sections contained granulomatous inflammation surrounding colorless round to ovoid structures, most consistent with *Prototheca* sp. Phaeophycomycosis is an infection with dematiaceous (pigmented) fungi which are widely distributed mycotic agents that contain melanin in their hyphae and conidia. *Prototheca* are achlorophyllic saprophytic algae that are also ubiquitous in the environment. The concurrence of mycotic and algal infections has been previously reported in equines and as with this case suggests that the animal may have been immunocompromised.

### **LB-21: ADRENAL GLAND OSSEOUS METAPLASIA AND MYELOLIPOMA IN ALASKAN POLAR BEARS (URSUS MARITIMUS)**

David Rotstein, Raphaela Stimmelmayer, Billy Adams, Mike Pederson

**Background:** Subsistence harvest of marine mammals by Alaskan natives is exempted under the Marine mammal Protection act (1972). Post mortem evaluation of subsistence harvested marine mammals provides an opportunity to better understand background infectious exposures and species specific incidental lesions. Polar bear (*Ursus maritimus*) which inhabits aquatic and terrestrial environs presents an important arctic key-stone species for examination for natural history information.

**Objective:** Present histopathologic findings from the adrenal glands of subsistence hunted Southern Beaufort Sea polar bears.

**Methods:** Paired adrenal glands from 78 polar bears were collected, fixed in formalin, sectioned at 5 to 7  $\mu\text{m}$ , and stained with hematoxylin and eosin..

**Results:** Thirteen polar bears had focal to multifocal osseous metaplasia, myelolipomas, or both in the adrenal gland. Individual, unrelated animals had focal osseous metaplasia in the kidney and lung. Osseous metaplasia, which was characterized by deposition of mature bone in the cortex, was observed in 6/13. Myelolipomas with deposition of mature adipocytes and myeloid cells was observed in 3/13 animals. Myelolipomas and osseous metaplasia was observed in 4/13 animals. Overall adrenal lesion incidence was 13/ 78 (17%).

**Conclusions:** The findings in the adrenal gland are considered incidental. However with fluctuating environmental changes and habitat changes, monitoring population health and background lesions can aid in assessing population responses.

## **LB-23: NEURAL NETWORKS AND DEEP LEARNING TO DEVELOP ALGORITHMS FOR AUTOMATED IMAGE ANALYSIS OF THYROID HYPERTROPHY AND HYPERPLASIA**

Zhewei Wang, Wendy Cai, Noriko Kantake, Jundong Liu, Thomas Rosol

**Background:** Image analysis of histopathology is typically performed manually or semiquantitatively using commercial software that depends on a restricted ability to segment structures. Neural networks and deep learning have the potential to train computer algorithms for efficient complex pattern recognition and measurement of histopathological changes.

**Objective:** To develop automated algorithms for the quantitation of thyroid follicle area, colloid area, and follicular epithelial cell height and number using digital scans of hematoxylin & eosin-stained rat thyroid glands.

**Methods:** Training of the automated algorithms was conducted using 'ground truth' data from manual image analysis (QuPath) of the scans. A patch segmentation solution was created as a fully functional convolutional network adopted from U-Net. Modifications to U-Net were made to match the ground truth data and tasks, which included use of a different padding scheme and adaptive channels for the final output layer. Dice loss was employed as the objective function to the data imbalance issue.

**Results:** A model algorithm (Follicle-Seg-Net) was created. The segmentation results were generated by an ensemble of three Follicle-Seg-Nets, which captured follicular cells (ring-net) and follicle and colloid boundaries (boundary-nets), respectively. Ring-net results were used as masks to optimize the segmentations obtained from two boundary-nets. The machine learning algorithm was rapid and compared favorably with ground truth measurements (~95% accurate; Dice = 0.90). Analysis of additional ground truth data will improve accuracy of the algorithm.

**Conclusion:** A machine learning approach was effective at generating a rapidly employed algorithm to automatically quantitate the morphology of rat thyroid follicles.

#### **LB-24: EVALUATION OF HISTOCHEMICAL TECHNIQUES FOR THE ASSESSMENT OF AIRWAY EPITHELIAL MUCINS**

David Meyerholz, Amanda Beck, J. Goeken, Mariah Leidinger, Georgina Ofori-Amanfo, Hannah Brown, Thomas Businga, David Stoltz, Leah Reznikov, Heather Flaherty

Histochemical stains are a foundational tool for histopathological assessment of mucin in the airway tree of the lung as well as in other tissues. Periodic acid Schiff (PAS) and Alcian Blue (AB) are common histochemical stains used to evaluate neutral and acidic mucins; however, nonspecific staining by tissue factors (e.g. glycogen) could influence mucin evaluation. We evaluated pig tracheas (<1 week of age, n=8) using PAS and AB techniques in relation to serial sections of diastase-pretreated PAS (dPAS) stained sections. Digital images were evaluated using CellSens Software (Olympus) and percent of the surface epithelium staining in serial sections was compared using a nonparametric paired t-test in relation to dPAS staining. The PAS technique had a statistically significant elevation in positive staining compared to dPAS samples ( $P=0.0078$ ), whereas the AB technique showed no significant differences to dPAS samples ( $P=0.9999$ ). Two pathologists evaluated tissues for detection of mucin and nonspecific background staining in dPAS vs. PAS or dPAS vs AB groups. No statistically significant differences were seen in comparisons except for elevated nonspecific background scores in PAS vs. dPAS techniques ( $P=0.0156$ ). Our results suggest that in the airway surface epithelium, the presence of glycogen can significantly influence the clinical and quantitative evaluation of mucus staining. For optimum specificity to mucin, preferential use of dPAS over PAS staining techniques would be beneficial in lung studies (and for other mucosal tissues) as a mucus marker.

#### **LB-25: EFFECT OF CYTOKINES ON MYXOMA VIRAL REPLICATION IN CANINE CANCER CELLS**

Bryce Zietz, Laura Ashton, Amy MacNeill

Cytokines exhibit a plethora of effects on cells including the admittance or blockage of viral replication. Certain cytokine combinations may be effective in altering cell permissiveness, rendering cells non-permissive to viral infection. We know that some cancer cell types allow myxoma virus replication whereas normal non-rabbit cells do not. We wish to investigate why this is and if canine cancer cells respond similarly to certain cytokine combinations used in human or mouse cancer cell studies. Based on prior research into the effects of cytokines in blocking myxoma virus replication in

human and mouse cancer cell lines, we hypothesize that canine cancer cells will respond similarly to human cancer cells. To test this hypothesis, we conducted a series of ELISAs to observe which cytokines healthy canine cells secrete when infected with virus. We are also studying the cytopathic effects of virus-infected cells when exposed to different cytokine concentrations or combinations. Currently the study shows canine cancer cells respond unlike mouse or human cancer cells altogether. Ongoing studies will give us additional insight into which cytokines are involved in permitting myxoma virus infection in canine cancer cells. This information will be invaluable for allowing a more successful targeted viral oncolytic therapy in cancer patients.

#### **LB-26: NOVEL MORBILLIVIRUS IN A FRASER'S DOLPHIN (*LAGENODELPHIS HOSEI*) FROM HAWAII**

David Rotstein, Kristi West, Nelmarie Landrau Giovannetti, Thomas Waltzek

**Background:** Marine mammal strandings are a normal occurrence, but strandings of some species, such as Fraser's dolphins (*Lagenodelphis hosei*) are infrequent to rare necessitating a thorough necropsy and ancillary diagnostics.

**Objective:** Present gross, histopathologic, and molecular findings.

**Methods:** A 2.044 m, 183.5 kg adult male Fraser's dolphin (*Lagenodelphis hosei*) stranded dead in Hawaii in early 2018 was necropsied.

**Results:** The dolphin was in good body condition with cookie cutter shark bites and intra-specific rake marks on the skin. There was focal blubber hemorrhage. Microscopic findings included multi lymph node and splenic lymphoid depletion with germinal center necrosis and rare syncytical cells, alveolar histiocytosis and focal fibrosis with rare syncytical cells, and portal hepatitis with bile duct intranuclear inclusions. There was a mild lymphocytic meningitis. PCR for cetacean morbillivirus (CeMV) and *Brucella* was conducted on brain, lung, liver, and multiple lymph nodes (left hilar, mediastinal, right prescapular). *Brucella* was negative. All tissues were positive for cetacean morbillivirus. Considerable sequence variation was detected between this Fraser's dolphin CeMV and CeMV sequences available for comparison in public databases, including a beaked whale morbillivirus previously described from Hawaii. A novel cetacean morbillivirus could be circulating in this population, but given no mass mortality events as observed in cetaceans in the Atlantic and Gulf, the impact is not known.

**Conclusions:** Fraser's dolphins rarely strand in Hawaii with the last report in 2006, but continued efforts to monitor and test stranded Fraser's dolphins could assist with prevalence and population health effects.

#### **LB-27: PHYLOGENETIC ANALYSIS OF AVIAN POXVIRUSES USING THE CORE PROTEIN 4B GENE IN NORTH AMERICAN AVIAN SPECIES**

Amanda MacDonald, Daniel Gibson, John Barta, Rebecca Poulson, Justin Brown, Andrew Allison, Nicole Nemeth

**Background:** Avian poxvirus is a highly contagious viral pathogen of both wild and domestic birds. These viruses are found globally; however, host-specificity related to genetic differences in avian poxviruses needs further exploration and a broader

understanding of the phylogenetic relationships is important to aid in determining strain-related pathogenicity.

**Objectives:** We performed a phylogenetic analysis to compare avian poxvirus isolates from species in Ontario and the United States to isolates previously derived from birds in six continents to determine strain relatedness. To our knowledge, this is the first phylogenetic analysis of avian poxviruses including birds from both Canada and the United States.

**Methods:** PCR analysis targeting the 4b core protein was used to amplify poxvirus DNA from 82 birds of varying species. Following sequencing, a phylogenetic analysis was performed that included these new 82 isolates and 70 publicly available sequences from GenBank.

**Results:** Phylogenetic results suggest four distinct clades among sequences analyzed. The majority (n = 57; 69.5%) of birds in this study were wild turkeys and 55 (96.5%) clustered with the fowlpox reference strain. Rare clustering of isolates with reference strains not characteristic of the host occurred. For example, one (1.2%) wild turkey clustered with mourning dove isolates and one (1.2%) clustered with the canarypox reference strain.

**Conclusions:** Most new isolates examined clustered with their respective poxvirus reference strain, suggesting poxvirus infections are often taxonomically restricted. Sporadic incidences of inter-specific poxvirus infections, however, demonstrate evolving strains that harbor the potential to infect more than one host species.

## **LB-28: INCIDENTAL PITUITARY ADENOMAS IN TWO SQUIRREL MONKEYS (SAIMIRI SCIUREUS)**

Gregory Daggett Jr, Jennifer Wood, Sanjeev Gumber, Christopher Pinelli

Two geriatric male captive squirrel monkeys (*Saimiri sciureus*) were found to have pituitary masses as incidental findings on post-mortem examination. Both animals were euthanized due to unrelated clinical reasons. At gross necropsy, the pituitary tumor in case 1 was approximately 3-4 mm in diameter that partially obscured and replaced the optic chiasm; while the tumor in case 2 revealed pituitary enlargement within the sella turcica, approximately 0.5 cm in diameter. Histologically, both tumors had similar morphologic characteristics but differing growth patterns. The tumor from case 1 was invasive into the adjacent neuropil and optic nerves, while the tumor from case 2 was expansile and compressing the adjacent neuropil. Neoplastic cells in both tumors were arranged in nests and packets supported by fine fibrovascular stroma with no mitoses detected. Based on immunohistochemical staining for thyroid stimulating hormone, adrenocorticotrophic hormone, growth hormone, follicle stimulating hormone, and luteinizing hormone these tumors were classified as thyrotroph (case 1) and corticotroph (case 2) pituitary adenomas. Both tumors were considered to be non-productive, as neither animal had clinical signs or gross pathologic correlates for hyperthyroidism or hyperadrenocorticism, respectively. Pituitary adenomas are rare in

nonhuman primates, and have previously been reported in cynomolgus and rhesus macaques, baboons, and chimpanzees. These are the first spontaneously reported cases of this tumor type in squirrel monkeys.

#### **LB-29: ARTIFACTS RESULTING FROM THE USE OF NEW PAINTBRUSHES TO PREPARE BONE MARROW SMEARS**

Rebecca Armstrong

Bone marrow smears that were examined as part of a training session contained a large quantity of variably sized and shaped angular, hyaline to granular or fibrillar material that was clear to bright magenta in color with Wright's Giemsa stain. The presence of the material was associated with cell lysis that was extensive enough to preclude evaluation of the smears, prompting an investigation. The material was determined to have originated from the paintbrushes used to prepare the bone marrow smears, which had recently been purchased in bulk directly from the supplier (as opposed to singly from the supplier's retail outlet) and had either never been used or had only been used to prepare a few bone marrow smears. A similar artifact was present in smears prepared from a different type of paintbrush, indicating that the contaminant is present in paintbrushes from multiple manufacturers. Thorough cleaning of new brushes before use eliminated the artifact.