TRYPANOSOMA THEILEIRI INFECTION IN A CALF FROM INDIANA

A.P. Santos¹, P.L. Deshuillers¹, N.C. Nascimento¹, S.D. Taylor², A.W. Bianco², J.B. Messick¹, and C.T. Thompson¹
¹Department of Comparative Pathobiology, Purdue University, College of Veterinary Medicine, West Lafayette, Indiana, USA, ²Department of Veterinary Clinical Sciences, Purdue University, College of Veterinary Medicine, West Lafayette, Indiana, USA

An eleven-day-old, female Simmental calf from Indiana was referred to the Purdue University Veterinary Teaching Hospital in February 2015 for decreased nursing. On presentation, the calf was depressed, dehydrated, and had a body temperature of 97.1ºF. CBC revealed severe anemia (PCV: 6.8%), mild mature neutrophilia and lymphopenia. Upon blood smear evaluation, organisms consistent with Trypanosoma spp. were observed. To explore the possibility of vertical transmission, blood from the calf’s mother was collected and conventional PCR (cPCR) for the cathepsin L-like gene was performed in both samples. Amplified PCR products were directly sequenced. Both specimens were cPCR positive. Sequencing revealed 100% identity with Trypanosoma theileiri when compared to the GenBank database. The calf received a blood transfusion and milk replacer and was discharged after five days of nursing and weight gain, (PCV: 14.1%). T. theileri are the most widespread trypanosomes in cattle and are considered non-pathogenic. A few reports associate clinical disease and anemia to T. theileri infection, especially when immunosuppression, poor nutrition, and/or co-infections are present. Since no obvious source of the anemia was identified, it is likely that T. theileri may have contributed to its development; however, the presence of co-infections with other blood borne pathogens was not fully investigated. Transmission of these protozoa occurs mostly by insects, however, the low incidence of potential vectors during winter and the age of the animal suggest that the infection was transplacental or via milk. This report suggests that T. theileri infection should be considered an occasional pathogen of cattle.

HEMATOLOGICAL DISORDERS ASSOCIATED TO BARTONELLA SPP. IN SHELTER CATS FROM RIO DE JANEIRO, BRAZIL
Background: Cats are likely the main reservoir of Bartonella henselae, B. clarridgeiae and B. koehlerae, agents commonly associated to human and domestic animals disease. Studies reveal that infected cats rarely exhibit clinical and hematological abnormalities, although B. henselae infection has been implicated in uveitis, endocarditis, stomatitis and lymphadenopathy. In clinical veterinary practice, some cats demonstrate non specific clinical signs and the laboratory findings have not been well characterized. Objective: The aim of the study was to evaluate clinical signs and hematological disorders associated with bartonellosis upon detection of Bartonella spp. DNA in blood of naturally infected cats. Methods: Blood were collected from 144 cats from shelters of Rio de Janeiro state after a clinical evaluation. Complete blood counts were performed. The presence of Bartonella spp. was screened by 16S-23S rRNA Intergenic Spacer region and gltA gene by conventional PCR. Results: Of the 144 samples, 50 (34.7%) were positive for Bartonella spp. The most common clinical conditions in positive cats were lymphadenopathy (48%), upper respiratory tract disorder (32%), conjunctivitis (10%), sporotrichosis (10%) and fever (6%). Eosinophilia was statistically associated to the infection and was observed in 72% of Bartonella spp. positive cats. Although there was no association to infection, polycythemia, lymphocytosis and neutrophilia were observed frequently in positive cats. Conclusion: Veterinarians should ponder Bartonella spp. infection in those cats with eosinophilia of unknown cause, as well as differential diagnosis of some diseases that share no specific clinical manifestation.

**Poster Number:** C-3

**Section:** Clinical Pathology

**Keyword:** Infectious Disease

**TWO DOGS INFECTED WITH BABESIA SP. (COCO) IN OKLAHOMA**

S. Fujita, L.A. Starkey, R.W. Allison, and J.H. Meinkoth
Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK

Large Babesia spp. infecting dogs are morphologically indistinguishable. Historically, all were classified as B. canis but have since been re-classified into three species: B. canis, B. rossi, and B. vogeli. Babesia sp. (Coco) is another large, morphologically indistinguishable Babesia species, first identified in 2004 in a Labrador retriever from North Carolina being treated for lymphoma. Since then, it has been identified in several dogs from North Carolina and Texas. Recently, we identified two dogs from Oklahoma infected with this new large Babesia sp. Dog 1 presented to Oklahoma State University with bloody/mucoid diarrhea. She had a history of splenectomy 5-6 years prior and one week duration of steroid treatment for back pain two weeks previously; travel history was unknown. A CBC revealed marked thrombocytopenia (Platelets: <20,000/µl) with a normal HCT. Dog 2 was being treated for lymphoma by a private practitioner; travel history was unknown. A CBC submitted to our laboratory revealed moderate anemia (HCT: 26%), severe thrombocytopenia (Platelets: <20,000/µl), and moderate neutropenia (Neutrophils: 1,558/µl). Blood films from both dogs showed erythrocytes containing large piroplasms (3-5µm) varying in shape from round to oval to pyriform, consistent with large Babesia spp. Whole blood samples from each dog were evaluated by nested PCR (18S rRNA gene); resultant amplicon
sequences were ≥99.3% similar to Babesia sp. (Coco) sequences previously reported in GenBank. These findings expand the reported geographic range of this organism. Immunosuppression, including chemotherapy or asplenia, is suspected as a risk factor for infection with Babesia sp. (Coco).

Poster Number: C-4

Section: Clinical Pathology
Keyword: Infectious Disease

MOLECULAR SURVEY OF MYCOPLASMA SPP. IN DOGS FROM SHELTERS OF RIO DE JANEIRO STATE, BRAZIL USING REAL TIME PCR

Federal Rural University of Rio de Janeiro (UFRRJ), Rio de Janeiro, Brazil

Background: Hemoplasmas are epierythrocytic parasites which have been reported in several mammalian species, including dogs. Clinical cases of hemoplasmosis in dogs have occasionally been reported, in which infections may lead to hemolytic anemia. However, most of the dogs remain chronic carriers. In Brazil the studies are scarce, especially those that focus on detection and molecular characterization of Mycoplasma spp., as well as its importance in clinical practice. Objective: The aim of this study was to detect Mycoplasma spp. in dogs belonging to shelters from Metropolitan Region of Rio de Janeiro using Real-Time Polymerase Chain Reaction (qPCR) as diagnostic tool and evaluate hematological abnormalities and risk factors associated with the infection. Methods: 130 samples were collected from dogs in shelters located in the municipality of Seropédica, Cacarica, Campo Grande and Guapemirim, state of Rio de Janeiro. Data were collected with a questionnaire on gender, race, age, presence of other pets in the house, place of residence, contact with other animals. Complete hematological analysis and qPCR for the detection of Mycoplasma spp. based on the gene encoding 16S rRNA were performed. Results: From all samples analyzed, 20.77% (n = 27) were positive by qPCR. Anemia was associated to the infection (p<0.05) and it was observed in 44% of positive dogs. None of the other factors were associated with the infection. Conclusion: Mycoplasma spp. is present in dogs from Metropolitan Region of Rio de Janeiro and should be considered a potential cause of anemia in clinical practice.

Poster Number: C-5

Section: Clinical Pathology
Keyword: Clinical Pathology

BIOLOGICAL VARIATION, INDEX OF INDIVIDUALITY AND REFERENCE CHANGE VALUE OF HEMATOLOGICAL AND BIOCHEMICAL VARIABLES IN LABORATORY CATS

C. Trumel, C. Monzali, A. Geffré, D. Concordet, L. Hourqueig, J.P. Braun, and N. Bourgès-Abella
Université Toulouse, INP, Ecole Nationale Vétérinaire de Toulouse, UMS006 INSERM-UPS, Laboratoire Central Biologie Médicale, Toulouse, France
Interpretation of clinical pathology results is usually based on the medical context and on positioning of patient’s results with respect to population-based reference intervals (RI) in which analytical, interindividual and intraindividual variability overlap. Indeed if intraindividual variability is much smaller than interindividual variability, subject-based RI or the reference change value (RCV) will be more sensitive to detect changes over time in an individual. The aim of this study was to document the biological variation of hematological and biochemical variables from laboratory cats for which homogeneity of living conditions should minimize between-subject variability. Blood specimens from 14 overnight-fasted laboratory cats sampled seven times for 3 months were analysed for routine hematology and biochemistry variables. For each variable, analytical, intraindividual and interindividual CVs were estimated prior to calculation of the index of individuality and the RCV. RBC count, HGB, HCT, MCV, MCH, MCHC, RDW, cholesterol, creatinine, and ALP exhibited marked individuality. Glucose, sodium and chloride had low individuality. The other variables (reticulocytes, WBC, neutrophil, lymphocyte, monocyte, eosinophil, platelet counts, albumin, total proteins, triglycerides, urea, ALT, AST, CK, calcium, carbon dioxide, iron, magnesium, phosphate, and potassium) had high to intermediate individuality. Subject-based RI or RCV would be preferable to monitor RBC variables and indexes, cholesterol, creatinine, and ALP in laboratory cats. Population-based RI are adequate for glucose, chloride and sodium, and both types of RI are similarly efficient for the majority of biochemical analytes, WBC and platelets.

Poster Number: C-6

Section: Clinical Pathology

Keyword: Hematology

REACTIVE LYMPHOCYTOSIS IN A BEARDED DRAGON

B. Meyer, K. Fisher, L. Corriveau, and R. Raskin
Comparative Pathobiology, Purdue University, College of Veterinary Medicine, West Lafayette, IN

Background: A ten-month-old male bearded dragon presented for pale mucous membranes. Serial complete blood counts (CBC) and biochemistry panels were performed over three months to follow a moderate to marked lymphocytosis (55,800/ul with RI: 4,000-12,000/ul), initially diagnosed as lymphoid leukemia. The patient also had intestinal parasite infections (Isospora and Pharyngodon spp.) on presentation that resolved with treatment. Objective: This case demonstrates problems using automated cell analyzers for reptile blood and available methods used to distinguish similar appearing nucleated cells such as lymphocytes and thrombocytes. Methods: Peripheral blood smears stained with Modified Wright and periodic acid-Schiff (PAS) were evaluated for differential and estimated cell counts. Since the hematology analyzer (Cell Dyn 2700) gave inaccurate leukocyte counts, 200 cells were used for the differential counts and 50 fields at 500x were used for estimated cell counts. Statistical analysis determined the mean, standard deviation, coefficient of variation (CV) and 95% confidence intervals. Results: Single-blinded lymphocyte estimates of sequential samples showed a decrease from 55,800/ul to 14,600/ul over the three months that corresponded to the treatment and elimination of the Isospora sp. infection. Lymphocytes were best distinguished from thrombocytes using cytomorphology and PAS stain. CD3 epsilon, CD79a, BLA.36, CD41/CD61, and vWF immunostaining did not assist in distinguishing the two populations. Conclusion: Marked lymphocytosis was attributed to the parasitic infection rather than a lymphoid leukemia. Automated analyzers have difficulty
CHANGES IN HEMOSTATIC TESTS IN DOGS WITH CHRONIC KIDNEY DISEASE

D.S. Gonçalves, S.S. Geraldès, P.T.C. Guimarães-Okamoto, and R.K. Takahira
School of Veterinary Medicine and Animal Science, Sao Paulo State University (Unesp), Botucatu-SP, Brazil

Chronic kidney disease (CKD) can predispose dogs to thrombotic or bleeding conditions. Azotemia and proteinuria may cause hemostatic changes such as hypercoagulability. Therefore, the aim of this study was to evaluate hemostatic changes in dogs with CKD. Fourteen dogs with CKD (IRIS stage III and IV) and urine protein-to-creatinine (UPC) ratio >1 were compared to 20 controls. Coagulation profile of all dogs was assessed by prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, thromboelastometry (in-TEM, ex-TEM and fib-TEM activators; Rotem® - Delta) and whole blood platelet aggregation (Chrono-log® - 592). Dogs with CKD presented a shortening of clot formation time (p<0.001), increased maximum clot firmness (p<0.001), α angle (p<0.001) and lower maximum lysis (p<0.001) with ex-TEM, in-TEM and fib-TEM activators. When evaluating the intrinsic pathway, neither the clotting time with in-TEM reagent (p=0.61) nor the aPTT (p=0.20) presented statistical difference. On the other hand, PT presented lower (p<0.001) values for dogs with CKD. Fibrinogen was higher in the CKD group (p<0.001). Platelet aggregation did not present statistical difference (p=0.07), despite the lower platelet aggregation values in CKD dogs. In conclusion, they presented signs of hypercoagulability which may be considered a higher thrombotic risk. Nevertheless, the platelet may not have a role in these changes. Further studies are needed to evaluate the clinical significance of these findings and the necessity of therapeutic interventions.

BLOOD GAS ANALYSIS IN CATS: A RETROSPECTIVE STUDY OF THE CLASSIFICATION OF COMMON DISORDERS IN DOMESTIC FELINES

Department of Veterinary Clinical Pathology, Veterinary Teaching Hospital, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Background: Blood gas analysis may provide information about the role of acid-base balance in the diagnosis and follow-up of several disorders that affect domestic cats, besides giving information about
Background: Urine microscopy has traditionally been a manual process requiring skilled technicians to prepare the sample and interpret the images from the microscope. Recently, a human analyzer, 77 Elektronika UriSed 2, with automated microscopy has been marketed to automate the process and improve accuracy and reproducibility of urine formed elements identification. Performance is based on training a neural network with technician-confirmed element images. To date no similar analyzer has been marketed for veterinary samples. Many formed elements will be very similar between human and veterinary samples, but some, like erythrocyte size, may require species-specific features. Objective: To evaluate a novel veterinary automated urine microscopy approach, the IDEXX SediVue DxTM, for identifying formed elements in urine. Methods: An automated inverted microscopy system with dedicated cuvette and on-board centrifugation has been developed and will be described from a function and imaging perspective for urine microscopy. Manual microscopy captured images at 400 x magnification from an inverted microscope of neat urine were compared to images from the automated SediVue microscopy system. Results: Visual comparisons of manually captured and automated captured microscopic images were determined to be of equal quality for manual identification of RBCs, WBCs, bacteria, epithelial cells, casts and crystals. Images from the automated capture system were consistent in quality regarding contrast and focus. Conclusions: The automated nature of the SediVue results in images of consistent quality that may lead to more consistent diagnostics over manual methods. Follow-

Poster Number: C-9

Section: Clinical Pathology
Keyword: Clinical Pathology

APPLICATION OF CURRENT AUTOMATED URINE MICROSCOPY IN VETERINARY MEDICINE

D.B. DeNicola¹, J. Hammond¹, G. Bayer², G. Bilbrough¹, A. Rebar³, and R. Cowell¹
¹IDEXX Laboratories Inc., Westbrook, ME, USA, ²77 Elektronika Kft, Budapest, Hungary, ³Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, West Lafayette, IN, USA

Objective: To evaluate a novel veterinary automated urine microscopy approach, the IDEXX SediVue DxTM, for identifying formed elements in urine. Methods: An automated inverted microscopy system with dedicated cuvette and on-board centrifugation has been developed and will be described from a function and imaging perspective for urine microscopy. Manual microscopy captured images at 400 x magnification from an inverted microscope of neat urine were compared to images from the automated SediVue microscopy system. Results: Visual comparisons of manually captured and automated captured microscopic images were determined to be of equal quality for manual identification of RBCs, WBCs, bacteria, epithelial cells, casts and crystals. Images from the automated capture system were consistent in quality regarding contrast and focus. Conclusions: The automated nature of the SediVue results in images of consistent quality that may lead to more consistent diagnostics over manual methods. Follow-
on studies will be completed to evaluate the performance of the veterinary-specific classification algorithms.

**Poster Number:** C-10

**Section:** Clinical Pathology  
**Keyword:** Clinical Pathology

**ERYTHROCYTE AND LEUKOCYTE COUNT PRECISION WITH AN IN-CLINIC AUTOMATED URINE SEDIMENT MICROSCOPY SYSTEM**

J. Hammond, L. Moisan, G. Bilbrough, and D.B. DeNicola  
IDEXX Laboratories Inc, Westbrook, ME, USA

Background: Urine microscopy has traditionally been a manual process requiring skilled technicians to prepare the sample and interpret microscopic images. A new automated urine microscopy system, the IDEXX SediVue DxTM, is under development for veterinary sample in-clinic application. The system requires 170 µL of neat urine to be transferred into a disposable cuvette, the sample is centrifuged and the system analyzes digital microscopic images resulting in a report of urine formed elements. Kova Urine Sediment controls are used to confirm performance.  

Objective: To evaluate the five-day CLSI between-run precision of the SediVue using Kova sediment controls.  

Methods: Kova controls are well-established for RBC and WBC microscopic evaluation; they have excellent stability of greater than 12 months. Two morning and two afternoon runs of control levels 1 and 2 were made on two different SediVue prototypes for five consecutive days yielding 20 total runs on each analyzer. The mean, standard deviation and %CV for RBC and WBC were determined. Results: Level 1 had <3 cells/HPF and the statistics have little value since no or few cells were found in most fields of view. For level 2 RBC, the mean, SD and % CV were 40/HPF, 5/HPF and 11% for instrument 1 and 39/HPF, 3/HPF and 9% for instrument 2. For WBC, the results were 34/HPF, 3/HPF and 9% for both instruments. Conclusions: Precision was excellent for RBC and WBC, especially when compared to standard manual urine microscopy with reported within-run %CV values on the order of 30-40%.

**Poster Number:** C-11

**Section:** Clinical Pathology  
**Keyword:** Clinical Pathology

**EXAMINATION OF IMPRECISION AND EFFECTIVENESS OF DIFFERENT CENTRIFUGATION AND UNCENTRIFUGATION METHODS FOR URINE SEDIMENT MICROSCOPIC EVALUATION**

J. Chase, J. Hammond, G. Bilbrough, and D.B. DeNicola  
IDEXX Laboratories Inc, Westbrook, ME, USA

Background: Urine microscopic evaluation is an essential component of a complete urinalysis and it is fundamental to health screening. In veterinary medicine, various urine microscopy methods are utilized...
in veterinary practices as well as academic and commercial laboratories. Centrifugation is the primary method used to prepare urine samples, but there are some methods that do not utilize centrifugation and documentation regarding similarities and differences between the different methods is needed. Objective: To document the imprecision of four urine microscopic evaluation methods. Methods: Two centrifugation and two non-centrifugation methods were compared to one another. Method A was 5 mL urine centrifuged at 390 g for 5 minutes. Method B was 1.5 mL urine centrifuged at 3900 g for 45 seconds. Method C was 60 µL neat urine evaluated by inverted microscopy in the flat bottom well of a 96-well microtiter plate. Method D was 30 µL neat urine on a slide with a coverslip. A suspension of canine erythrocytes was prepared for a 10-replicate within-run precision assay for all methods. Results: The coefficient of variation (mean/SD) for RBC counts/HPF were 52% (55/29), 71% (23/17), 14% (64/9) and 48% (14/7), respectively for methods A, B, C and D. Conclusion: Based on these results, centrifugation does not appear to provide the perceived amplification benefit and there is significant variability for all sample preparation methods. Regarding precision, the microtiter plate method with a neat sample performed the best (%CV of 14%) and the 1.5mL centrifugation method performed the worst (%CV of 71%).

Poster Number: C-12

Section: Clinical Pathology
Keyword: Infectious Disease

EVALUATION OF (1,3)-BETA-D-GLUCAN AS A BIOMARKER OF SYSTEMIC FUNGAL INFECTION IN DOGS AND CATS

J. Renschler and L.J. Wheat
MiraVista Diagnostics, Indianapolis, IN

The laboratory diagnosis of systemic fungal infections is often challenging and requires assessment of multiple testing modalities including cytology/histopathology, fungal culture, antigen tests on urine and/or serum and antibody tests. Additional biomarkers of fungal infection will help to support a diagnosis when the results of other diagnostics are equivocal or false negative. (1,3)-beta-D-glucan (BDG) is a cell wall polysaccharide component found in most fungi. The objective of this study was to evaluate the utility of BDG as a biomarker of systemic fungal infection in dogs and cats. BDG was assessed in serum by using a commercial assay (Fungitell, Associates of Cape Cod, MA, USA) in dogs and cats with proven histoplasmosis (Hc; n=27), coccidioidomycosis (Ci; n=27), systemic aspergillosis (As; n=22) and other systemic fungal infections (n=4; including penicilliosis, paecilomycosis and uncharacterized mold infections) as well as healthy controls (n=59) and clinical controls (n=112). Sensitivity was 96% for Hc, 44% for Ci, 77% for As and 100% for other fungal infections. BDG was positive in 3 cases with negative Ci antigen, as well as 2 As cases with negative galactomannan antigen. Specificity was 88% in healthy controls and 72% in clinical controls. BDG appears to show clinical utility as an adjunctive diagnostic test for systemic fungal infections; however, further investigation of causes of false positives in clinical and healthy controls is needed.

Poster Number: C-13

Section: Clinical Pathology
Keyword: Hematology
EVALUATION OF A CHROMOGENIC ASSAY TO MEASURE THE FACTOR Xa INHIBITORY ACTIVITY OF LOW MOLECULAR WEIGHT HEPARIN IN RABBIT PLASMA

F. Poitout-Belissent¹, L. Huard¹, M. Fergusson-Graton¹, D. Lourdel¹, J. McCartney¹, T. Nekoroski², and R. Sekulovich²
¹Charles River Laboratories- Montreal, Senneville, QC, Canada, ²Halozyme Therapeutics, San Diego, CA, USA

Background: Potential drug interference/interaction with Low Molecular Weight Heparins (LMWH) may be required for specific drug safety evaluations. Prolongation of activated partial thrombin time, typical with heparin administration, does not provide sufficient specificity for a potential drug-heparin evaluation. LMWH inhibits activated factors II and X through binding of anti-thrombin (AT). Assays measuring inhibition of activated factor Xa are commonly used to monitor patient safety. Objective: We evaluated the suitability of a chromogenic anti-Xa activity assay (STACHROM® Heparin, Diagnostica Stago) on a STA ® Compact System for monitoring LMWH activity in rabbits. Methods: Heparin present in rabbit plasma binds to a bovine AT reagent. Bovine factor Xa and a chromogenic substrate for factor Xa are then added, and the heparin-AT complexes inhibit factor Xa. The quantity of chromogenic substrate released (detected colorimetrically) is inversely proportional to the heparin concentration. The standard curve is derived using known high and low concentrations of Tinzaparin®, a commercially LMWH, spiked in normal pooled rabbit plasma as calibrators. Quality control is performed by assaying LMWH in human plasma (STA® HBPM/LMWH controls). Results: Measurement range: 0 to 1.20 UI/mL. Precision: intra-assay coefficient of variation (CV): 0.8 to 1.4%, inter-assay CV: 2.1 to 2.9% Accuracy using controls: CV: 1.5 to 2.5%. Linearity of dilution range: 0.08 to 1.02 UI/mL. Stability: up to 1 day at room temperature, 7 days at -80°C. Conclusion: the STACHROM® Heparin assay accurately measures LMWH heparin anti-Xa activity in rabbit plasma, and is suitable for the detection of potential drug-LMWH interactions in preclinical studies.

Poster Number: C-14

Section: Clinical Pathology
Keyword: Clinical Chemistry

ASSESSMENT OF TWO GLUCOSE METERS FOR GLYCEMIC CONTROL IN HORSES

Department of Veterinary Clinical Pathology, Veterinary Teaching Hospital, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

Background: The use of handheld human glucose meters in horses allows for the quick measurement of glucose levels; however, it is necessary to assess their performance to prevent inaccurate results. Objective: The aim of this study was to evaluate the performance of two handheld glucose meters (Contour TS – Bayer, and AccuCheck Active – Roche) for the measurement of glucose levels in horses,
using error grid analysis (EGA) and following ISO 15197:2013 recommendations. Methods: Twenty horses were included in the study. Their blood was collected in EDTA (hematocrit) and fluoride (glucose by colorimetric method) tubes, and a drop of fresh blood was used to measure glucose levels in both devices. The results were assessed by EGA. Results: Hematocrit (HCT) levels ranged from 23 to 40%, but eight horses had an HCT below 29%. Contour-TS yielded 85% of accuracy within zone A (analytical precision), and 15% of accuracy within zone B (a deviation of more than 20% from the reference value, which could lead to changes in treatment). Conversely, AccuCheck Active yielded an accuracy of 100% within zone A. Conclusion: According to ISO 15197:2013, a glucose meter can be considered to be accurate if 100% of its results are within zone A and B of the error grid; therefore, both meters are accurate and can be used in horses, despite HCT variations. Nonetheless, AccuCheck may be more advantageous as it minimizes the risk of treatment errors.

**Poster Number:** C-15

**Section:** Clinical Pathology

**Keyword:** Endocrine

**EVALUATION OF A HUMAN GLYCATED HEMOGLOBIN TEST FOR THE DIAGNOSIS OF DIABETES MELLITUS IN DOGS**

N-Y Kim¹, M-C Kim¹, G-W Ha², J-H Kim², S-W Yeh², and H. Kim³

¹Laboratory of Clinical Pathology and BK 21 PLUS Program for Creative Veterinary Science Research, College of Veterinary Medicine, Seoul National University, Seoul, The Republic of Korea, ²BioNote, Inc., Hwaseong-si, Gyeonggi-do, The Republic of Korea, ³Haemaru Referral Animal Hospital, Seongnam City, Gyeonggi-do, The Republic of Korea

Glycated proteins, fructosamine and glycosylated hemoglobin (GHb), widely used to monitor diabetes mellitus in humans. Their amount is proportional to the blood glucose concentration for a long period and not affected by transient hyperglycemia. In dogs, fructosamine has been preferred over GHb to assess glycemic control because more analytical assays for fructosamine are available that are less time-consuming and uncomplicated. Recently it has shown that rapid kits for human GHb recognize the canine counterpart. This study was performed to evaluate the potential value of a human GHb test kit for the diagnosis and monitoring of canine diabetes mellitus. EDTA-treated whole blood samples from 110 dogs and 30 diabetes mellitus dogs were tested using the POC instrument. It clearly differentiated normal dogs from hyperglycemic dogs. The test had high sensitivity and high specificity for diagnosing diabetes mellitus. The test was simple, easy to perform and required only 5 ul of whole blood. The results were highly reproducible with very low coefficient value. The concentrations were mildly affected by anemia. Considering that fructosamine assay is more expensive and availability of the reagents is limited in some countries, the GHb test would provide more options for the veterinary clinicians.

**Poster Number:** C-16

**Section:** Clinical Pathology

**Keyword:** Immune System
SOLUBLE CD163 (sCD163), A NOVEL BIOMARKER OF MACROPHAGE ACTIVATION AND PROLIFERATION IN CYNOMOLGUS MONKEYS

M. Winter, F. Christen, E. Atzpodsien, J. Funk, F. Regenass, and M. Odin

CD163, an endocytic receptor for haptoglobin-hemoglobin complexes is expressed at low levels on macrophages and monocytes, with significantly increased expression on activated type-2 macrophages. A shedded subunit of the receptor circulates in normal serum as a soluble protein (sCD163). Elevated serum sCD163 concentrations have been shown to correlate with an expanded and activated macrophage population. An assay for cynomolgus monkey sCD163 was established on the Gyros Platform and analytically validated against human sCD163. Crossreactivity to cynomolgus CD163 was demonstrated in a series of tests with cynomolgus and human serum samples. Groups of cynomolgus monkeys received a 24-hour intravenous infusion of a novel drug candidate at 0, 100 and 400 mg/kg/day once every 4 days as for a total of 6 cycles. Clinical pathology samples were taken at various time-points and histopathology and immunohistochemistry examination was performed at study termination. Markedly elevated concentrations of sCD163 were found in cynomolgus monkey serum samples at study termination with increasing concentrations seen already after the first infusion cycle in the 400 mg/kg dose group. Liver histopathology revealed Kupffer cell hyperplasia/hypertrophy and increased numbers of intravascular monocytes with a foamy cytoplasm and high CD163 expression, indicating activation and expansion of type-2 macrophages. Serum sCD163 concentrations correlated with the histopathologic findings and proved to be a sensitive and selective biomarker for type-2 macrophage activation and proliferation.

Poster Number: C-17

Section: Clinical Pathology
Keyword: Clinical Chemistry

THE DNA-ALKYLATING, ANTICANCER DRUG TEMOZOLOMIDE PROTECTS RATS FROM DOSE-LIMITING PANCREATIC TOXICITY PRODUCED BY THE SMAC-MIMETIC, ANTICANCER DRUG TL3271

Z. Zakaria¹, P.J. O’Brien², M.F. O’Brien², A. Byrne¹, and J.H.M Prehn¹
¹Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, ²Advanced Diagnostics Laboratory, University College Dublin

Combination of the anticancer agents temozolomide and smac-mimetics (SM) has synergistic efficacious effects in vitro, but their toxicity in combination is unknown. Accordingly, in repeat-dose, rat, toxicity studies we compared separate versus combined toxic effects of SM and TMZ. SM antagonise inhibitor-of-apoptosis proteins of neoplastic cells and induce NF-κB thereby increasing TNFα-mediated inflammation and hepatotoxicity, and dose-limiting pancreatic toxicity. Temozolomide (TMZ) is an alkylating, agent with mild myelotoxicity that causes autophagy of glioblastoma cells. We used glutamate dehydrogenase (GLD) to assess hepatotoxicity, reticulocytes to evaluate myelotoxicity, and the novel, pancreatic-specific biomarker DGGR-lipase to evaluate pancreatic toxicity. Sprague-Dawley (8 week old) rats were assigned to 4 groups (n=5 per group): vehicle, TMZ, SM, TMZ and SM. Tail vein
blood was taken prior, during and after treatment. We administered 25 mg/kg TMZ daily for 7 days and 20 mg/kg SM (TL3271) 3 times a week for 3 week. After SM dose 5 or 6, no SM rats survived and the treatment in the combination group was halted after 2 weeks. SM rats had increased neutrophils (up to 3-fold), and serum GLD (up to 5-fold) and DGGR-lipase (up to 30-fold), which were not seen in the combination group. TMZ decreased reticulocytes by three-quarters, neutrophils by one-third, and lymphocytes by one-half. We conclude that in the rat the pancreatic toxicity of SM is dose-limiting, and can be detected using the novel pancreatic biomarker DGGR-lipase, and that this toxicity and hepatotoxicity detected by GLD is prevented by temozolomide.

**Poster Number:** C-18

**Section:** Clinical Pathology  
**Keyword:** Clinical Pathology

**SENSITIVITY AND SPECIFICITY OF THE PCR-BASED LYMPHOCYTE CLONALITY ASSAY (PARR) FOR THE DIAGNOSIS OF B- AND T-CELL LYMPHOMA IN CATS**

S.E. Hammer¹, S. Groiss¹, A. Fuchs-Baumgartinger², N. Nedorost³, N. Luckschanger-Zeller³, S.M. Keller⁴, A. Saalmüller¹, I. Schwendenwein⁵, and B.C. Rütgen⁵  
¹Institute of Immunology, ²Institute of Pathology, ³Clinic for Internal Medicine, VetmeduniVienna, Austria, ⁴Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, UCDavis, USA, ⁵Clinical Pathology Unit, VetmeduniVienna, Austria

With an incidence of 200 per 100.000 individuals lymphoma is the most common hematopoietic neoplasia in cats. Diagnostic classification of infiltrates consisting of well differentiated small lymphoid cells is often challenging and the differentiation between a resident mature lymphocyte population and small cell lymphoma cannot be made by cytology alone. These cases warrant the application of complementary tools like PCR-based immunoglobulin and T-cell receptor clonality testing (PARR) for confirmation. In this study, we evaluated diagnostic sensitivity and specificity of the PARR assay with specified primer sets for routine diagnosis of feline TCR gamma (TCRG) and complete IG heavy chain (IGH) V-J gene rearrangements. Cytology samples from 20 histologically confirmed feline lymphoma cases were evaluated. Lymph node material from 10 cats without hematopoietic neoplasia served as negative controls. The feline lymphoma cell lines MS4 and FT-1 and histological confirmed patient material were used as positive controls. To assess clonality, different primer sets were compared in triplicate PCRs followed by size separation of the PCR products by capillary electrophoresis. Diagnostic sensitivity and specificity of the assay were 90% and 89%, respectively. Overall diagnostic accuracy was 89%, the PPV 95% and the NPV 72%. The PARR clonality assay differentiates between a monoclonal and a polyclonal lymphoid population and can be used as a reliable complementary tool for the confirmation of feline lymphoma. Nevertheless, present data are only valid for these particular primer sets used in this study.

**Poster Number:** C-19

**Section:** Clinical Pathology  
**Keyword:** Hematology
FIRST REPORT OF CIRCULATING DOUBLE POSITIVE CD4/CD8 T LYMPHOCYTES IN A CAT WITH THYMOMA

P.L. Deshuillers, A.P. Santos, and R.E. Raskin
Department of Comparative Pathobiology, Purdue University College of Veterinary Medicine, West Lafayette, Indiana, USA

Background: An 8 year-old domestic shorthair was referred to the oncology service following difficulty breathing. Results: Upon admission, a CBC revealed a moderate leukocytosis (43.4 x 10^3/µL) associated with a moderate to marked lymphocytosis (34.3 x 10^3/µL) and a moderate basophilia (1.3 x 10^3/µL). On blood smear examination, the majority of these cells were small lymphocytes. Immunophenotyping revealed 11.9% of lymphocytes co-expressing CD4 and CD8 (4 x 10^3/µL). Upon radiographic and ultrasonographic examinations, a mass occupying approximately 80% of the chest was found and aspirated. The specimen had low nucleated cellularity requiring histologic evaluation for a definitive diagnosis. Surgery attempted mass removal and obtained a surgical biopsy sample. Unfortunately, during surgery the cat went into cardiac arrest and could not be resuscitated. Histologic evaluation of the mass identified a thymoma with an abundant infiltration by small lymphocytes. Discussion: The thymoma could be the cause of the lymphocytosis after most of the other causes have been excluded by clinical, hematology, biochemistry and bone marrow evaluations and a negative PARR assay.

Lymphocytosis is a rare paraneoplastic syndrome associated with thymoma and multiple phenotypes (CD4/CD8 double positive or double negative T lymphocytes) have already been documented. The mechanism remains unclear and a spill-over of lymphocytes from the thymus or thymopoietic hormones could be involved. Additionally, double positive CD4/CD8 T lymphocytes have recently been identified in healthy individuals or presenting with a variety of disease (i.e., auto-immune). This is the first report of circulating double positive CD4/CD8 T lymphocytes in a cat.

Poster Number: C-20

Section: Clinical Pathology
Keyword: Clinical Pathology

CONCURRENT MULTIPLE MYELOMA AND MAST CELL NEOPLASIA IN A THIRTEEN-YEAR-OLD CASTRATED MALE MAINE COON CAT

J.M. Bagwell, H.R. Herd, M.A. Breshears, and T.E. Rizzi
Oklahoma State University Veterinary Pathobiology

Case Description: A thirteen year old CM Maine Coon cat presented to Oklahoma State University BVMTH for yearly echocardiographic examination monitoring hypertrophic cardiomyopathy (HCM) diagnosed in 2003. Physical examination revealed a heart murmur and premature beats, likely related to HCM; but was otherwise unremarkable. Routine blood work revealed a hyperglobulinemia [6.3g/dL (2.3-5.3)]. Clinicopathologic Findings: Serum protein electrophoresis with immunofixation confirmed an IgG monoclonal gammopathy. Liver and spleen cytology contained increased numbers of plasma cells and mast cells confirmed with subsequent histologic examination. Immunohistochemistry (IHC) for cKit in the spleen showed mast cells predominantly exhibiting type I staining pattern with moderate numbers exhibiting type II pattern. Within the liver, IHC for cKit showed mostly mast cells with type I staining and
scattered cells exhibiting type II and III patterns. Bone marrow cytology and core biopsy contained approximately 22% plasma cells. Additional cutaneous masses on patient’s left shoulder and right carpus were cytologically confirmed mast cell tumors. Relevance: This is an example of two hematologic neoplasms occurring simultaneously in a patient. Concurrent pathologies are unusual and may be overlooked if a single disease is the presumed cause of clinical signs. Both neoplasms were well differentiated and could have easily been interpreted as a reactive population if full workup had not been performed. Undetected and untreated, either neoplasm could have led to the death of the patient. Treatment and Outcome: The patient was splenectomized and received chemotherapy. Seven months post diagnoses, the patient was clinically well, and globulins decreased to 5.4g/dL.

**Poster Number:** C-21

**Section:** Clinical Pathology  
**Keyword:** Neoplasia

**APPLICATION OF HEMOSTATIC TESTS IN THE THROMBOTIC RISK IN DOGS WITH MAMMARY CARCINOMA**

R.K. Takahira, T.C. Trentin, D.S. Gonçalves, and C.Z. Garcia  
School of Veterinary Medicine and Animal Science, São Paulo State University (Unesp), Botucatu-SP, Brazil

**Background:** Mammary tumors are considered the most common tumor in dogs and can often predispose them to hemostatic complications secondary to neoplasia. In this context, thrombosis is a major hemostatic abnormality in patients with cancer, which is often underdiagnosed. Hemostatic tests have been used in human medicine for an earlier diagnosis, allowing a better prognosis of neoplastic conditions. However, tests to diagnose hypercoagulable states are rarely used in dogs with cancer. In veterinary medicine the correlation between cancer and hypercoagulability has been a constant finding in dogs, but most of the times, it is devoided of clear clinical signs. The need for hemostatic evaluation to establish a better treatment protocol and prognosis in different types of cancer is evident in these patients. **Objective:** To evaluate the hemostatic profile and the possible hypercoagulable states in dogs with mammary carcinoma. **Methods:** Fifteen female dogs with mammary carcinoma confirmed by cytology and histopathology where compared to 15 female healthy dogs (control group). Both groups were submitted to tromboelastometry (INTEM, EXTEM, FIBTEM), platelet aggregation, fibrinogen, PT and aPTT. **Results:** The results were compared by the t test at the level p<0.01 and p<0.05. There were significant differences in hemostatic routine tests resulting in increased platelet aggregability and prolonged PT. Tromboelastometry had demonstrated a significant shortening in CT, CFT, and ML and an increase in α angle and MCF for both EXTEM and FIBTEM reagents. **Conclusion:** We conclude that dogs with mammary carcinoma present a higher thrombotic risk associated to a hypercoagulable state.

**Poster Number:** C-22

**Section:** Clinical Pathology  
**Keyword:** Wildlife
MOLECULAR CHARACTERIZATION OF AN INTRAERYTHROCYTIC ORGANISM IN AN ANEMIC LOUISIANA BLACK BEAR

S. Dehghanpir, Y. Sokolova, J. LaCour, K. Banajee, and B. Grasperge
Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA

In January 2015, the Louisiana Department of Wildlife and Fisheries discovered an orphaned, four-month-old Louisiana black bear (Ursus americanus luteolus) with a right shoulder degloving injury. The cub was febrile (104°F), in poor body condition (~20 kg), and infested with ticks that were not identified. Pulmonary auscultation revealed diffuse harsh sounds over the left lung field. A complete blood count showed moderate regenerative anemia (HCT 18%, Retic 3.9%) with small (~1.5-2.5 µm diameter), intraerythocytic, ring-form Babesia-like organisms observed on a modified-Wright’s stained blood smear. Ultrastructural morphology, PCR amplification, and sequencing of whole blood were utilized to identify the organism. The nucleotide sequences of the 18S rRNA gene had 99% identity to sequences from several unclassified Babesia spp., including isolates from Japanese black bears. Treatment with tetracycline (400 mg subcutaneously) for pneumonia was initiated and ameliorated clinical signs. To the authors’ knowledge, babesiosis in a North American black bear has not previously been reported.

Poster Number: C-23

Section: Clinical Pathology
Keyword: Wildlife

OCCURRENCE OF HEMOPLASMOSIS IN WILD CAPTIVE DOGS AND CATS FROM THE FEDERAL DISTRICT, BRAZIL

F.T. Carneiro¹, G. Amorim¹, M.C. Scaloni¹, L.C. Aquino¹, F.M.A.M. Pereira², B.P. Borges², and G.R. Paludo¹
¹Laboratório de Microbiologia e Patologia Molecular, Hospital Veterinário, Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília, Brazil, ²Fundação Jardim Zoológico de Brasília, Brasília, Brazil

Background: Haemoplasmosis are of great importance in veterinary clinic of domestic animals given the severity of clinical signs, depending on the parasite species and host immune competence. However, data on its epidemiology in wildlife medicine are not much clear. Objective: This study intended to investigate which haemoplasma species parasitize wild carnivores at Federal District in order to clarify the epidemiology of the disease in the region. Methods: Blood samples were collected from 18 wild canines and 34 wild felines from Fundação Jardim Zoológico de Brasília and conservationist breeding facility NEx and sent to the Molecular Microbiology and Pathology Laboratory of the Veterinary Hospital of University of Brasília. DNA extraction was performed with Illustra blood genomicPrep Mini Spin Kit. Subsequently, PCRs were conducted with primers 5'-ATACGGGCCCCATATCTCAG-3' and 5'-TGCTCCACACTTGTCCA-3' for general haemoplasma detection, 5'-GACTTTGGTTTCGGCCAAGG-3' and 5'-CGAAGTACTATCATATTCCCTCC-3' for Mycoplasma haemofelis detection, 5'-GCTAGTCTGCAATCTTCAT-3' and 5'-GGTTCAACTAAGTTTCTCC-3' for Candidatus Mycoplasma haemominutum detection, and 5'-GTCCTTAGTATCCTCCATACAGACAG-3' and 5'-CGACACATTGTACTCCACTTGTAA-3' for Candidatus Mycoplasma turicensis detection. Results: From the tested specimens, 27 were positive for haemoplasmas, 16 canines and 11 felines. Interestingly, no animals were positive for M. haemofelis, 4
were positive for C. M. haemominutum (3 canines and 1 feline) and 6 were positive for C. M. turicensis (5 canines and 1 feline). Conclusion: The most recurrent hemoplasma species in domestic animals are not the most frequent in wild carnivores. Candidatus M. haematoparvum, M. haemocanis, or even new species are probably more likely to appear. Besides, canines seem to have a more important role as reservoir of the disease.

**Poster Number:** C-24

**Section:** Clinical Pathology  
**Keyword:** Wildlife

**BONE MARROW CYTOMETRY OF HEALTHY ADULT COATIS (NASUA NASUA) RAISED IN CAPTIVITY**

R.K. Takahira, D.S. Gonçalves, B.P. Monteiro-Stegall, and L.M. Ozeki  
School of Veterinary Medicine and Animal Science, Sao Paulo State University (Unesp), Botucatu-SP, Brazil

Coatis (Nasua nasua) are carnivorous animals, vastly distributed through Americas with a zoonotic potential. The purpose of this study was to evaluate the bone marrow cytology of coatis (Nasua nasua). Bone marrow aspirate samples were aspirated from the proximal femur in a 3% EDTA saline solution from five male adult and three female healthy coatis under anesthesia. Bone marrow was expelled in a petri dish and the particles were collected by a hematocrit capillary tube to make at least five good quality air dried slides. Cytomorphology and myeloid to erythroid (M:E) ratios were evaluated in 500 nucleated cells. The mean ± SD cellularity was 30.6 ± 20.2% with a M:E ratio of 1.89 ± 0.5. The mean ± SD percentage of immature erythroid, mature erythroid, immature myeloid, mature myeloid, monocytoid, eosinophilic, lymphocytes, and plasma cells were 2.37 ± 1.4, 24.9 ± 5.5, 2.85 ± 1.4, 46.6 ± 5.6, 0.0 ± 0.1, 8.1 ± 2.4, 12.2 ± 3.3, 3.0 ± 2.3, respectively. Most of the megakaryocytic cells were mature and were found in a mean of 6.25 ± 1.3 cells per large particle. All hematopoietic cell lines were represented in adequate number and showed orderly maturation. The cellularity was lower than the expected for other domestic carnivores such as dogs and cats, however it was not possible to establish the age of the individuals. Bone Marrow evaluation in coatis has shown to be a simple procedure that can be helpful for hematologic studies in this species.

**Poster Number:** C-25

**Section:** Clinical Pathology  
**Keyword:** Wildlife

**SERUM BIOCHEMISTRY OF ADULT FREE-LIVING DIDELPHIS ALBIVENTRIS**

J.Y. Shimono, D.S. Gonçalves, F. Fornazari, and R.K. Takahira  
School of Veterinary Medicine and Animal Science, Sao Paulo State University (Unesp), Botucatu-SP, Brazil
Didelphis albiventris, the white-eared-opossum, is a marsupial found in the east and midwest of Brazil, Paraguay, Uruguay, Argentina and Bolivia with an important zoonotic potential as reservoir hosts for leptospirosis, trypanosomiasis, leishmaniasis and toxoplasmosis. The purpose of this study was to evaluate the serum biochemistry of white-eared-opossums (Didelphis albiventris) and the possible sexual influence on it. Blood samples were collected from 40 adult clinically healthy free-living Didelphis albiventris, twenty-one females and nineteen males, in Botucatu city, Sao Paulo State, Brazil. Urea, creatinine, total serum protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), phosphatase alkaline (ALP), gama-glutamyl transferase (GGT), calcium and phosphorus were analysed by Roche – Cobas Mira Plus chemistry analyzer. The results from males and females were compared by t test with a 5% level of significance. The mean ± SD results of urea, creatinine, albumin, ALT, AST, GGT, ALP, calcium and phosphorus were 77.26 ± 38.83 mg/dL, 0.43 ± 0.3 mg/dL, 2.96 ±0.30 mg/dL, 12.35 ± 4.87 U/L, 77.07 ± 38.01 U/L, 11.13 ± 5.72 U/L, 367.18 ± 163.67 U/L, 7.69 ±2.13 mg/dL, 7.04 ± 2.41 mg/dL for males 79.71 ± 51.84 mg/dL, 0.27 ± 0.3 mg/dL, 2.87 ± 0.34 mg/dL, 11.53 ± 6.02 U/L, 72.63 ± 55.52 U/L, 11.88 ± 8.70 U/L, 238.81 ± 107.73 U/L, 7.37 ± 1.55 mg/dL, 6.32 ± 1.28 mg/dL for females, respectively. Only ALP was significantly lower (p<0.05) in females than males. These results may be used as guidelines for assessing hematology of Didelphis albiventris in clinical and research studies.

**Poster Number:** C-26

**Section:** Clinical Pathology  
**Keyword:** Clinical Pathology

**EFFECT OF SUPPLEMENTATION WITH VITAMIN E ON MALONDIALDEHYDE LEVEL IN EQUINES SUBMITTED TO DIFFERENT INTENSITIES OF EXERCISE ON A TREADMILL HIGH-SPEED**

T.S. Barbosa, L.A. Yonezawa, C.L. Marinho, J.L. Knaut, M.J. Watanabe, and A. Kohayagawa

Background: During physical exercise there is an increase in oxygen consumption as much tissue all causing an overproduction of free radicals. When this generation exceeds the antioxidant capacity of cellular and extracellular fluids, injuries develop due to oxidative stress installed, resulting in damage and lipid peroxidation of cell membranes and tissue injury. Objectives: The objective was to evaluate the effect of supplementation with vitamin E in horses submitted to two types of exercise on high-speed treadmill, through the determination of lipid peroxidation. Methods: Ten untrained horses performed two exercise tests, high intensity (HI) and low intensity (LO). The HI test consisted of 5 min at 50% VO2max, 5 min at 1,5 min and 90 s at 105% VO2max set at +6% slope, and 5 min at 3,0 m/s (0% slope). The LO test consisted of 60 min at 35% VO2max (+6% slope). Plasma malondialdehyde (MDA) was determined by HPLC at rest before exercise, immediately after exercise, 6 h, 12 h and 24 h after test end. The animals were supplemented with dl-α-tocopherol at a dose of 1,000 UI/day during 45 days and then they performed the second test with the same protocol. Results: It was observed that supplementation with vitamin E reduced plasma concentrations of MDA at all times of HI and only at 24 h of LO. Conclusions: Therefore, we conclude that the efficacy of vitamin E was higher in the exercise of low intensity long duration.

**Poster Number:** C-27
TECHNICAL VALIDATION AND BIOLOGIC QUALIFICATION OF SERUM IRON HOMEOSTASIS BIOMARKERS IN JUVENILE RATS AND MONKEYS

Drug Safety Research and Development, Pfizer Inc, Cambridge, MA

The objective of this study was first to validate a panel of serum iron biomarkers (iron, total iron binding capacity [TIBC], transferrin, ferritin and %transferrin saturation-calculated) and second, to retrospectively evaluate the temporal changes in serum iron biomarkers in well-characterized, archived nonclinical serum with potential iron overload in juvenile male and female rats and monkeys. Siemens Advia 1800 automated chemistry analyzer was used for technical validation (precision, dilutional linearity, recovery spiking, freeze thaw stability, limit of detection and biologic correlation) using appropriate calibrators, controls and reagents. Biologic qualification was assessed using well characterized serum samples with potential iron overload in juvenile rats and monkeys as evidenced by microscopic evidence of hepatic iron accumulation. Absolute quantitation of hepatic iron from the juvenile monkey samples was assessed by inductive coupled plasma-mass spectroscopy (ICP-MS). Results generated from serum samples during the hepatic iron accumulation phaseshowed serum iron, ferritin and % transferrin saturation elevations consistent with the microscopic and ICP-MS findings of hepatic iron accumulation. Mechanistically, increased serum iron concurrent with increased transferrin saturation reflected true serum iron increases as serum TIBC and transferrin were mostly unchanged. During recovery phase, there was partial to near complete recovery of serum iron and % transferrin saturation elevations while serum ferritin continued to be elevated when compared to the iron accumulation phase. In summary, technical validation and biologic assessment of a panel of serum iron biomarkers provided quantitative and mechanistic data to understand the temporal and molecular basis for hepatic iron accumulation in juvenile rats and monkeys.

A RUBRIC FOR EVALUATING PROCESSING ARTIFACTS IN HISTOLOGIC SPECIMENS WITH INKED MARGINS

P.K. Kiser¹, M. Milovancev², C.V. Löhr¹, and D.S. Russell¹
¹Department of Biomedical Sciences, Oregon State University, ²Department of Clinical Sciences, Oregon State University
Quantitative and semi-quantitative histopathologic margin assessment is widely used to evaluate adequacy of excision in surgical biopsy specimens. However, there is limited data on processing artifacts and their impact on margin evaluation. We hypothesize that processing artifacts in sections of normal skin can be identified with high inter- and intra-observer agreement. To test this hypothesis, we devised a novel rubric for quantifying the frequency of processing artifacts. One hundred and five sections of inked cadaveric canine skin were evaluated by two independent observers for tissue deformation (tissue deflection and contraction), ink-associated artifacts (incomplete inking, dissection along fascial planes, inappropriate ink distribution, inappropriate coloring), and sectioning artifacts (tissue folding, separated tissue, incomplete sectioning, floated tissue). Inappropriate ink distribution was most frequent (present in a mean of 68.1% of samples), and tissue folding was least frequent (6.7%). Both categories had moderate inter-observer agreement with a Cohen’s kappa coefficient of 0.41 and 0.39, respectively. Inappropriate coloring (22.4%) and separated tissue (38.1%) had modest inter-observer agreement with kappa coefficients of 0.54, and 0.44, respectively. Incomplete sectioning (50.0%) and tissue deflection (39.5%) had poor inter-observer agreement (k=0.19 and 0.02, respectively). There was substantial intra-observer agreement for tissue folding (mean k= 0.73) and dissection along fascial planes (k= 0.68); tissue deflection had only slight agreement (k= 0.14). Possible reasons for suboptimal observer agreement include overlapping categories that are indistinguishable, subjectivity within individual categories, and differences in observer proficiency. This rubric will be further refined, with the goal of prospectively applying it to clinical biopsy specimens.

**Poster Number:** D-2

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Neoplasia

**ACANTHOMATOUS AMELOBLASTOMA WITH ATYPICAL FOCI IN FIVE DOGS**

J. Malmberg, B. Powers, and P. Schaffer
Colorado State University, Fort Collins, CO

Acanthomatous ameloblastoma is a fairly common tumor of the canine oral cavity that is locally invasive but does not metastasize. A variant of acanthomatous ameloblastoma with atypical foci was noted in five dogs. There was no age, breed, or gender predisposition. Atypical cells were immunohistochemically negative for cytokeratin, vimentin, MelanA, PNL2, CD18, CD3, and Pax5. Complete excision was achieved by maxillectomy or mandibulectomy in three dogs; the lesion was incompletely excised in two dogs. No ancillary therapy was elected in any of the patients. Follow up time varied from 6 months to 2.5 years. No local recurrence or distant metastasis was reported in any case. Acanthomatous ameloblastomas with atypical foci do not appear to be associated with any greater risk of local recurrence or metastasis and are not associated with a poor prognosis relative to acanthomatous ameloblastomas with typical histologic morphology.

**Poster Number:** D-3

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Hematology
ADULT-ONSET, CYCLIC THROMBOCYTOPENIA IN A RHESUS MACAQUE

G.H. Frydman1,2,3, K. Pate4, R.P. Marini1, A.M. de Laforcade5, I. Bosch6, S. Muthupalani1, A.G. Swennes1, and J.G. Fox1
1Division of Comparative Medicine, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA, 2Division of Surgery, Science, and Bioengineering, Massachusetts General Hospital, Boston, MA, USA, 3Harvard Medical School, Boston, MA, USA, 4Department of Virology and Infectious Disease, Johns Hopkins University, Baltimore, MD, USA, 5Emergency and Critical Care, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA, 6Harvard-MIT, Health Sciences and Technology, Cambridge, MA, USA

Background: Diagnosis of platelet-based bleeding diatheses is often complicated and identification of causative etiology frequently elusive. Reports of thrombocytopenia in macaques (Macaca spp.) are rare and characterization of the associated platelet pathologies challenging because of the need for highly specialized training and equipment, the lack of species-specific reagents, and the time-sensitive nature of platelet function testing. Objective: In this report, a 14-year-old, male rhesus macaque (Macaca mulatta) previously used for dengue virus vaccine research with viral challenge, presented with adult-onset, chronic, cyclic thrombocytopenia. Methods: Platelet morphology and function were evaluated by peripheral blood smears, electron microscopy, flow cytometry, and impedance aggregometry. Bone marrow was evaluated by cytology. Serum anti-dengue non-structural protein 1 (NS1) antibodies were detected by ELISA. Results: Platelet characterization showed a lack of aggregation to all agonists (ADP, ASP, and collagen), increased activation with increased expression of surface markers (CD62P, CD40L, PAC1, HLA-ABC, HLA-DR), and an absence of surface receptor GPIIX during symptomatic episodes, even in the presence of normal platelet counts. Giant gray platelets were identified on peripheral blood smears, and electron microscopy revealed decreased alpha granules compared to controls during symptomatic episodes. Bone marrow aspirates identified mild megakaryocytic hypoplasia. All platelet functions and morphological attributes were within normal limits when the macaque was not symptomatic. Presence of anti-dengue NS1 serum antibodies confirmed a positive dengue virus titer 8 years post-vaccination. Conclusion: Based on the history and clinical findings, a primary differential for this chronic, cyclic platelet pathology is autoimmune platelet destruction with bone marrow involvement.

Poster Number: D-4

Section: Diagnostic Pathology
Keyword: Infectious Disease

AN OUTBREAK OF CYPRINID HERPESVIRUS-1 IN A BACKYARD KOI (CYPRINUS CARPIO) WATER GARDEN ASSOCIATED WITH THE STRESS OF RELOCATION DURING TEMPERATE CLIMACTIC CONDITIONS

N. Crossland, J. Hawke, Y. Sokolova, P. Viadanna, T. Waltzek, and F. Del Piero

Fifteen adult koi (Cyprinus carpio) developed multifocal, slightly raised-to-nodular, whitish cutaneous proliferations affecting up to 30% of the body. Development of cutaneous lesions correlated with recent relocation from a temporary pool to a remodeled backyard pond during the Louisiana fall season, were
average temperatures ranged from 13-27° Celsius. Cutaneous lesions histologically consisted of severe hyperplasia, dysplasia, full thickness keratinocyte apoptosis, keratinocyte hydropic degeneration, scattered goblet cells, and mild infiltration by lymphocytes and eosinophilic granule cells. Transmission electron microscopy of cutaneous biopsies revealed immature icosahedral nucleocapsids in the nucleus, with abundant mature enveloped virions within the cytoplasm. PCR targeting Cyprinid Herpesvirus-1 (CyHV-1) thymidine kinase gene was positive on formalin-fixed, paraffin-embedded skin sections. Cutaneous lesions resolved rapidly following administration of 2-3 ppm of rock salt in the coldest part of the year. These findings are consistent with an outbreak of CyHV-1, attributed to stress associated with relocation to a new environment. Rapid resolution of cutaneous lesions following viral latency is supportive of a hyperplastic rather than a neoplastic process, and the authors propose discontinuation of the terminology papilloma when referring to CyHV-1 associated cutaneous lesions.

**Poster Number:** D-5

**Section:** Diagnostic Pathology

**Keyword:** Nervous System

**BILATERALLY SYMMETRICAL ENCEPHALOMALACIA IN A DWARF CAIMAN (PALEOSUCHUS PALPEBROSUS)**

W.M. Zoll¹, S.P. Terrell¹,², B.A. Stacy¹, L.L. Farina¹, D.J. Heard¹, M. Bercier¹, D.C. Honeyfield³, and J.R. Abbott¹

¹College of Veterinary Medicine, University of Florida, Gainesville, Florida, ²Animal Care, Health, and Science; Disney's Animal Kingdom, Bay Lake, FL, ³US Geological Survey, Northern Appalachian Research Lab, Wellsboro, PA

A 4-year-old male dwarf caiman (*Paleosuchus palpebrosus*) was evaluated by the Zoologic Medicine Service at the University of Florida Veterinary Hospital because of a six-week history of neurologic clinical signs that included depression, mild ataxia, head trembling and difficulty prehending food. A clutch mate housed with this animal showed similar but less severe signs. The caiman was euthanized and submitted for necropsy. There were no significant gross findings. Histopathologic examination of the brain was consistent with bilaterally symmetrical vacuolation (status spongiosis) and rarefaction of the neuropil of the white and gray matter of the myelencephalon, diencephalon and telencephalon. Other histologic findings included neuronal necrosis, chromatolysis and endothelial cell hyperplasia associated the capillary prominence. Small to moderate numbers of macrophages and fewer heterophils infiltrated the affected areas. A toxicologic, metabolic or hereditary neuropathologic etiology was suspected based on the history, clinical signs, gross and histologic examination. Ancillary diagnostics performed included blood lead, thiamine and vitamins E and A levels. Vitamin E and A levels were decreased in reference to serum vitamin E and A levels from six other crocodilians on the farm. Skeletal muscle and liver thiamine levels and serum lead levels were normal compared to published values in vertebrates and other crocodilians sampled from the farm. Hypovitaminosis E is suspected as the cause of the pathologic findings based on serum and histopathologic findings.

**Poster Number:** D-6

**Section:** Diagnostic Pathology

**Keyword:** Neoplasia
BOVINE PAPILLOMAVIRUS DNA AND S100 PROTEIN PROFILES IN
SARCOID AND OTHER EQUINE SPINDLE CELL TUMORS

E.D. Epperson and W.L. Castleman
Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida,
Gainesville, FL

Current diagnostic guidelines are that equine cutaneous spindle cell tumors positive for bovine
papillomavirus (BPV) DNA by PCR and negative for S100 protein by immunohistochemistry be diagnosed
as equine sarcoi d. Deep dermal and subcuticular sarcoi ds (i.e., nodular sarcoi d) pose a particular
diagnostic challenge since while epidermal involvement may be lacking, they may have
histomorphologic features difficult to distinguish from peripheral nerve sheath tumors (PNSTs) and
other spindle cell neoplasms including fibrosarcoma. Archived paraffin-embedded equine cutaneous
spindle cell tumors (n=198) received at the University of Florida between 1995 and 2013 were
histomorphologically characterized and evaluated for S100 protein immunoreactivity and for the
presence of BPV type 1 or 2 DNA by PCR. BPV DNA was detected in 49 of 70 (70%) sarcoi ds, 19 of 32
(59%) PNSTs, 20 of 54 (37%) fibrosarcomas, and 9 of 42 (21%) other spindle cell tumors (myxosarcomas,
fibromas, and other sarcomas). S100 protein immunoreactivity was present in only 12 tumors (5
fibrosarcomas, 3 sarcoi ds, 2 PNSTs, and 2 other sarcomas) . Our results demonstrate that BPV DNA is
present in many other skin associated spindle cell soft tissue tumors in horses in addition to sarcoi d, and
that S100 immunohistochemistry is of limited value in the diagnosis of equine cutaneous neoplasia.

Poster Number: D-7

Section: Diagnostic Pathology
Keyword: Nervous System

CAUSES OF CANINE MENINGOENCEPHALITIS AND MENINGOMYELITIS
IN GEORGIA: 129 CASES

W. Yau and D.R. Rissi
Department of Pathology and Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine,
University of Georgia, Athens, GA

Necropsy reports from dogs submitted to the Athens Veterinary Diagnostic Laboratory between 2008
and 2015 were reviewed for cases of meningitis, encephalitis, meningoencephalitis, myelitis,
meningoencephalomyelitis, and meningoencephalomyelitis. A total of 129 cases were retrieved and included in
this study. Infectious diseases were the most commonly diagnosed category (36% of the cases), with
diagnostic confirmation achieved by pathological changes, fluorescent antibody testing,
immunohistochemistry, and/or bacterial aerobic culture. Bacterial infections were the most prevalent
(17%) among the infectious diseases and consisted of Streptococcus spp., Staphylococcus spp., and E.
coli infection. Viral diseases (15%) consisted of canine distemper virus (9.3%), canine herpesvirus-1
(3.9%), and rabies virus (1 case) infection. One case of concurrent distemper and canine herpesvirus-1
infection was also confirmed. Rare infections included those caused by Cryptococcus neoformans (2
cases), Toxoplasma gondii or Neospora caninum, Prototheca sp., phaeohyphomycosis, and amebiasis.
(one case each). Non-infectious inflammatory conditions made up the second most common category of diseases (33% of the cases) and consisted of granulomatous meningoencephalitis (19%), necrotizing meningoencephalitis (9.3%), and steroid-responsive meningitis (3.9%). A definitive diagnosis was not reached in 31% of the studied cases. A viral or bacterial infection was suspected in 12% of these non-diagnostic cases, but confirmation failed due to negative results from ancillary testing or unavailability of fresh samples for testing. This study highlights the need to further characterize cases of neurological diseases of unknown cause in dogs by the use of additional diagnostic tests, such as immunohistochemistry and RNA sequencing using formalin-fixed paraffin embedded tissues.

**Poster Number:** D-8

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

### CD10, PAX8, AND NAPSIN-A AS IMMUNOHISTOCHEMICAL MARKERS OF CANINE RENAL CELL CARCINOMA

T.J. Peat¹, E. Edmondson², M.A. Miller¹, D.M. DuSold¹, and J.A. Ramos-Vara¹  
¹Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, ²Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO

CD10, PAX8, and Napsin-A are useful immunohistochemical markers of human renal cell carcinoma (RCC); however their diagnostic utility in canine RCC is unclear. Forty formalin-fixed, paraffin-embedded renal cell carcinomas (15 papillary, 12 solid, and 13 tubular) and ten metastases were evaluated for expression of CD10 (mouse monoclonal, clone 56C6, Biocare, Concord, CA), PAX8 (mouse monoclonal, clone BC12, Biocare), and Napsin-A (rabbit monoclonal, clone ACI3043, Biocare). Prolonged formalin fixation up to 28 days did not reduce CD10, PAX8 or Napsin-A expression in normal canine kidney. Immunoreactivity in tumors was scored as percent neoplastic cells with cytoplasmic/membranous (CD10), cytoplasmic (Napsin-A), or nuclear (PAX8) reactivity as 0 (<1%), 1 (1-15%), 2 (16-50%), or 3 (>50%). Thirty-nine (98%), 26 (65%) and 21 (53%) of the tumors expressed PAX8, Napsin-A, and CD10, respectively. PAX8 was expressed in 92% of solid, 100% of papillary, and 100% of tubular tumors. Napsin-A was expressed in 58%, 60%, and 62%, respectively. CD10 was expressed in 33%, 47%, and 62%, respectively. Among metastatic tumors, PAX8 was expressed in 80% of cases, Napsin-A in 60%, and CD10 in 40%. Overall, expression and intensity of reaction for each of these markers was heterogeneous within a given tumor. PAX8 immunoreactivity was stronger overall than that of Napsin-A or CD10. In summary, PAX8 is more sensitive than Napsin-A or CD10 for detecting primary and metastatic canine RCC; its nuclear and more intense reactivity also make it easier to interpret.

**Poster Number:** D-9

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia
COMPARISON OF IMMUNOHISTOCHEMICAL DETECTION OF IONIZED CALCIUM-BINDING ADAPTOR MOLECULE 1 (IBA1) WITH CD18 AND HLA-DR IN CANINE HISTIOCYTIC SARCOMA

S.A. Sokol and A.D. Miller
Section of Anatomic Pathology, Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Definitive diagnosis of canine histiocytic sarcoma (HS) usually requires phenotypic assessment of beta-2 integrin (CD18)-positive and lymphocyte antigen-negative neoplastic cells, but deficiencies in the specificity of CD18 for macrophage-lineage cells and variability of expression of CD18 in HS necessitate a search for more reliable markers of HS in the dog. Ionized calcium-binding adaptor molecule 1 (Iba1) recently has been shown to be a specific marker of microglia and other macrophage-lineage cells with usefulness in identifying histiocytic proliferative, neoplastic, and inflammatory disorders in the dog. We examined the immunoreactivity of a human polyclonal antibody to Iba1 and compared it with the expression of CD18 and human leukocyte antigen (HLA)-DR in 46 histiocytic sarcomas in 44 dogs diagnosed at Cornell University from 2011 to 2013. Anatomic location of HS varied in this study to include splenic (9/46), lymph node (8), hepatic (7), periartricular (6), cutaneous (4), central nervous system (4), intestinal (2), oral (2), bone marrow (1), pulmonary (1), and intramuscular (1) tumors. Forty-five of 46 (98%) tumors were positive for Iba1. Immunoreactivity was primarily strongly cytoplasmic with variable membranous and nuclear reactivity. Forty-three (93%) and 32 (70%) tumors expressed CD18 and HLA-DR, respectively. Intensity of expression of CD18 varied widely, and CD18 in particular displayed consistent non-specific immunoreactivity to neutrophils and necrotic debris. Intensity of expression of HLA-DR was predominantly weak or moderate. We conclude that Iba1 is a sensitive immunohistochemical marker for histiocytic sarcoma in the dog and more specific than CD18.

Poster Number: D-10

Section: Diagnostic Pathology Focused Group Session II
Keyword: Bone and Joint

ALKALINE PHOSPHATASE IMMUNOHISTOCHEMISTICAL STAINING TO DIFFERENTIATE OSTEOSARCOMA FROM OTHER PRIMARY BONE NEOPLASMS

K. Schlicher, A. Barger, E. Driskell, T. Fan, and K. Terio

Osteosarcoma (OSA) is the most common primary bone tumor in dogs. In tumors devoid of osteoid, histologic differentiation of OSA from other primary bone neoplasms such as chondrosarcoma (CSA), fibrosarcoma (FSA), and histiocytic sarcoma (HS) is challenging. Detection of alkaline phosphatase (ALP) activity via cytochemical analysis on bone tumor fine needle aspirates allows differentiation of OSA from other bone neoplasms but is not an applicable technique on formalin fixed tissue. This study examines the utilization of immunohistochemistry (IHC) for detection of ALP in primary bone tumors and its relative ability to differentiate OSA from other primary bone tumors. Cases of primary bone tumors with the diagnoses of OSA, CSA, FSA, and HS were retrieved from the University of Illinois Veterinary
Diagnostic Laboratory database and diagnosis confirmed. Immunohistochemistry using a tissue non-specific mouse monoclonal anti-ALP antibody as well as osteopontin and osteonectin antibodies were performed on 15 OSA, 4 CSA, 9 FSA, and 4 HS. Osteonectin and osteopontin IHC exhibited high sensitivity but low specificity. Positive cytoplasmic immunoreactivity for ALP was observed in all tumor types, with variable intensity and staining patterns both within and between tumor types. In six cases (2 FSA and 4 HS) the majority of the neoplastic cells either did not express or only displayed very faint cytoplasmic immunoreactivity. These results indicate that application of ALP IHC may not be useful in differentiating OSA from other primary bone tumor types, as it exhibits high sensitivity but low specificity.

**Poster Number**: D-11

**Section**: Diagnostic Pathology  
**Keyword**: Skin

**CONCURRENT CUTANEOUS PHAEOHYPHOMYCOSIS AND NOCARDIOSIS IN A DOG**

J. Bailey, J. Koehler, T. Hathcock, and A. White  
Auburn University College of Veterinary Medicine, Auburn, AL

A 6-year-old spayed female Doberman Pinscher with a previous diagnosis of pemphigus foliaceus presented with a two-week history of multifocal exudative and hemorrhagic skin nodules, cellulitis of the face and left hind carpus, and pitting edema of the left hind limb and right forelimbs. Microscopically, pigmented fungal hyphae were present within the lumen of hair follicles and the keratinized layer of the epidermis, as well as free within the dermis and subcutis. Also present within the dermis were scattered colonies of weakly Gram-positive, variably beaded, long filamentous rods. Both organisms were associated with multinodular pyogranulomatous inflammation. Curvularia sp., Nocardia sp., Streptococcus canis, and Staphylococcus intermedius were all isolated from the cutaneous lesions. This patient’s history of high doses of immunosuppressive drugs for treatment of pemphigus foliaceus likely played a role in development of this unusual multiorganism opportunistic infection.

**Poster Number**: D-12

**Section**: Diagnostic Pathology Focused Group Session I  
**Keyword**: Neoplasia

**CONCURRENT OCULAR T CELL LYMPHOMA WITH LINEAGE INFIDELITY AND HISTIOCYTIC SARCOMA WITH B CELL RECEPTOR IGH GENE CLONALITY IN A CAT (FELIS CATUS)**

K. Barnes, M. Kiupel, J.Stiles, M. Operacz, and D. Sledge

Enucleation was elected in a 17-year-old cat with a five-year history of suspected uveitis in the right eye that progressed to include corneal opacity obscuring intraocular structures. The globe was expanded
and ablated by two morphologically and immunophenotypically distinct neoplastic cell populations. Expanding and infiltrating the cornea and uvea was a smaller, monomorphic neoplastic round cell population that had immunoreactivity for CD3 and not CD20 or CD204, consistent with T cell lymphoma. Regionally expanding the stroma of the anterior uvea was a larger, anaplastic cell population that had immunoreactivity for CD204 and not CD3 or CD20, consistent with histiocytic sarcoma. PCR assessing clonal rearrangement of T and B cell receptor genes was performed on the histiocytic and lymphocytic populations separately following gross dissection from the formalin-fixed, paraffin-embedded tissue and laser capture microdissection. Clonal rearrangement was only confirmed for the B cell receptor IGH gene in both cell populations; however, the length of the clonal PCR products varied by 20 base pairs between the histiocytic and lymphocytic populations. Demonstration of clonality of the B cell receptor IGH gene in lymphocytes that are phenotypically T cell in origin is consistent with lineage infidelity. Clonality of the B cell receptor IGH gene in a histiocytic sarcoma may be due to transdifferentiation from a concurrent or previous lymphoma or sporadic inheritance of a B cell genotype. The different clonal peaks in the electropherograms support two clonally unrelated lymphocytic and histiocytic neoplastic cell populations and therefore suggest a unique histogenesis for each neoplastic population.

**Poster Number:** D-13

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Bone and Joint

**CONFIRMATION OF FIBRODYSPLASIA OSSIFICANS PROGRESSIVA IN A DOMESTIC SHORTHAIR KITTEN BY DETECTION OF THE R206H MUTATION IN THE ACTIVIN RECEPTOR 1A/ACTIVIN-LIKE KINASE-2 (ACVR1/ALK2) GENE**

M.D. Vieson¹, E.M. Shore², M. Xu², and G.K. Saunders¹

¹Dept of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, ²Dept of Orthopaedics, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA

Fibrodyplasia Ossificans Progressiva (FOP) is a rare, genetic disease in humans that results in the formation of extra-skeletal bone due to the progressive transition of soft connective tissues, including skeletal muscle and tendons, to endochondral bone. A domestic shorthair kitten presented with progressive immobility of joints of the appendicular skeleton starting at 10 weeks of age. Based on history, clinical signs, physical examination findings, radiographs, blood work, and pathological examination, FOP-like disease was diagnosed. To provide evidence to support a similarity of the disease in cats to FOP in humans, DNA was extracted from affected soft tissues around the joints and sequenced. As previously found in human FOP patients, a mutation in the activin receptor IA/activin-like kinase-2 (ACVR1/ALK2) gene was detected in the affected tissues from the kitten suggesting that the disease in cats is likely to be very similar to FOP in humans. The litter mates and dam are all healthy with no clinical signs of FOP at the time of publication of this report.

**Poster Number:** D-14
CRYPTOCOCCAL GRANULOMA AS A NEWLY REPORTED CAUSE OF CANINE JEJUNAL INTUSSUSCESSION

G. Krane\(^1\), C. Cummings\(^2\), and S. Craft\(^1\)
\(^1\)University of Florida College of Veterinary Medicine, Gainesville, FL, \(^2\)Ultrapath Imaging, Durham, NC

A 1.5 year old spayed Doberman Pinscher with a chronic history of loose stools presented for acute vomiting, diarrhea, and lethargy. Exploratory laparotomy revealed a jejunal intussusception. A resection and anastomosis was performed to remove the affected section, which was submitted for histopathology. Grossly, within the intussusception, there was a 4.5 x 3 x 2.5 cm firm, brown luminal mass connected to the intestinal mucosa via a 0.3 cm diameter stalk.

Microscopically, the mass was composed of ulcerated small intestine with abundant fibrous tissue and smooth muscle admixed with large aggregates of numerous 10-20 \(\mu\)m diameter, thin walled, round to oval yeast surrounded by a 5-10 \(\mu\)m thick clear capsule. The yeast retained PAS and mucicarmin histochemical stains, and they occasionally exhibited narrow based budding. Numerous macrophages, fibroblasts, and fewer eosinophils were admixed amongst the yeast. Multifocally in areas of necrosis within the intussuscipiens and intussusceptum were similar aggregates of yeast and inflammatory cells. Yeast were present at the orad and aborad surgical margins. This case was diagnosed as granulomatous and eosinophilic enteritis with intralesional fungi consistent with Cryptococcus sp. PCR and ultrastructural analysis are pending.

The literature regarding intestinal cryptococcosis in dogs is sparse. To our knowledge, this case represents the first reported case of an intussusception caused by a fungal granuloma, expanding the differential diagnosis list for this clinical entity.

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DIAGNOSTIC UTILITY OF CD204, CD163, AND IBA1 IN CANINE ARTICULAR HISTIOCYTIC SARCOMA AND SYNOVIAL CELL SARCOMA

K. Casey, V. Affolter, and P. Moore
University of California - Davis, Davis, CA

Histomorphologic distinction amongst tumors arising within the joints (intra-articular) and/or adjacent to the joints (peri-articular) of the canine appendicular skeleton are diagnostically challenging as they often overlap in their histomorphology and immunophenotype despite having significant variability in prognosis. Normal canine synovium is composed of synovial type A cells (phagocytic function) and synovial type B cells (synovial fibroblasts), the latter of which are considered to give rise to synovial cell sarcomas. Frequently, synovial cell sarcomas are infiltrated by a secondary population of histiocytes,
making distinction amongst synovial cell sarcoma (SCS) and periarticular/articular histiocytic sarcoma (p/aHS) difficult. Currently, no single immunohistochemical reagent, or panel of reagents, exists to definitively distinguish amongst these entities in formalin-fixed tissues, nor has the full immunophenotype of Type A synovial cells and subsynovial dendritic cells been fully elucidated. In this study, we use a set of 20 canine articular tumors (10 p/aHS and 10 SCS) and evaluate the histologic and immunohistochemical characteristics of normal and neoplastic synovium using the recently characterized antibodies to scavenger macrophage receptors (CD204, CD163) and lba1 (ionized calcium-binding adaptor molecule). Along with the antibodies CD1a, CD11b, and CD11c we expect to elucidate the characteristics of the canine synovium, the contribution of these cells to synovial sarcomas, and establish the diagnostic utility of the antibody panel in distinguishing canine SCS from p/aHS.

**Poster Number:** D-16

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**DISCORDANCE BETWEEN PARR AND HISTOPATHOLOGY/IMMUNOHISTOCHEMISTRY IN AN ATYPICAL LYMPHOCYTE POPULATION IN A CANINE GALLBLADDER**

G. Krane¹, S. Campos¹, C. Cummings², J. Abbott¹, and J. Struthers¹  
¹University of Florida College of Veterinary Medicine, Gainesville, FL, ²Ultrapath Imaging, Durham, NC

A 6 year old spayed female miniature dachshund presented for lethargy, vomiting, and inappetence. Following a diagnosis of cholelithiasis and pancreatitis, she developed septic and chemical peritonitis, leading to cholecystectomy with submission of gallbladder for histopathology.

Grossly, the gallbladder had a focal partial thickness defect and contained mucoid green liquid admixed with multiple 3-5 mm diameter irregularly-shaped, firm, black choleliths. Microscopically, the lamina propria was diffusely and severely expanded by an atypical population of sheets of monomorphic lymphocytes 7-11 μm in diameter. Anisocytosis and anisokaryosis were mild, and mitoses were 4-5/400x field. In adherent liver sections, this round-cell population multifocally infiltrated portal tracts. Lymphoma was diagnosed.

Immunohistochemistry for CD3 and CD79a followed. Approximately 65% of the round-cell population exhibited cytoplasmic CD79a immunoreactivity (B cells). In the deeper submucosa, comprising approximately 35% of the round-cell population, there were medium-sized, well-circumscribed, densely packed clusters of cells exhibiting cytoplasmic CD3 immunoreactivity (T cells). Neither B nor T cell antigen receptor clonality was confirmed by PCR. Ultrastructural analysis is pending.

Despite the incongruity of PARR results with histomorphology and IHC, we believe this represents the second reported case of canine gallbladder B-cell lymphoma, for which the first report lacked PARR. This case highlights the fact that PARR, though useful, cannot assess for all possible clonalities, and pathologists must also rely on contextual histologic and immunohistochemical interpretations.

**Poster Number:** D-17
LABEL-FREE SPERM MORPHOLOGY ASSESSMENT USING IMAGING FLOW CYTOMETRY

S. Vaidyanathan\textsuperscript{1}, A. Li\textsuperscript{1}, B.A. Didion\textsuperscript{2}, and P. Morrissey\textsuperscript{1}
\textsuperscript{1}EMD Millipore, Seattle, WA, \textsuperscript{2}MoFa Global, Verona, WI

Assessment of sperm morphology is one of the most important steps in the evaluation of the health of the spermatozoa. A higher percentage of morphologically abnormal sperm is strongly correlated to lower fertility. Morphological image analysis can be used to segment sperm morphology, extract associated quantitative features, and classify normal and abnormal sperm in large quantity. In the vast majority of fertility clinics and laboratories, brightfield microscopy is used to evaluate sperm morphology. However, since sperm morphology contains a variety of shapes, sizes, positions, and orientations, the accuracy of the analysis can be less than optimal as it is based on a limited number of cells (typically 100-400 cells per sample). To overcome this challenge, we used an ImageStream imaging flow cytometer to acquire brightfield and side-scatter images of porcine sperm samples at 60X magnification. We developed novel image algorithms to perform image segmentation in order to detect abnormal sperm cells using salient shape descriptors (invariant of scale, position, and orientation), such as diameter, circularity, elongation, corners, and negative curvatures. Taking advantage of the ability of the imaging flow cytometer to acquire images at a high resolution and speed, we demonstrate the validity of using image based parameters that can be adapted to each spectral image and features to assess sperm morphology in an objective and precise manner.

GRANULAR CELL VARIANT OF A CANINE ORBITAL MENINGIOMA: A CASE REPORT

G. Shaw, S.N. Miller, R.R. Dubielzig, and L.B.C. Teixeira

Comparative Ocular Pathology Laboratory of Wisconsin, University of Wisconsin, Madison, WI

Canine orbital meningiomas are rare tumors that arise from the arachnoid cap cells that surround the optic nerve. Meningiomas occur in several different histologic subtypes including the rare granular cell variant. Granular cell tumors are uncommon tumors of various histogenic origins characterized by PAS positive cytoplasmic granules that are identified as lysosomes on electron microscopy. This report describes an orbital meningioma with features characteristic of the granular cell variant in a dog. A 15-year-old spayed female dachshund presented with a one-year history of exophthalmos due to a retrobulbar mass. An exenteration was performed and the mass was submitted to COPLOW for evaluation where part of it was processed routinely for paraffin embedding and part of it was post-fixed in glutaraldehyde and then plastic embedded for electron microscopy. The tumor was composed of
large lobules of polygonal neoplastic cells with distinct cell borders, abundant finely granular amphophilic cytoplasm and eccentrically located round to oval nuclei. Cytoplasmic granules were rarely positive when stained with periodic acid Schiff (PAS) stain. Neoplastic cells reacted with antibodies to vimentin, but not to cytokeratin or neurofilament. Ultrastructurally the neoplastic cells presented irregular and interdigitating cytoplasmic extensions, desmosomal junctions, abundant variably sized membrane-bound cytoplasmic dense bodies containing electron-dense material and variable numbers of intermediate filaments. These histologic, immunohistochemical and electron microscopic features confirmed the diagnosis of a granular cell variant of a canine orbital meningioma.

**Poster Number:** Poster D-19

**Section:** Diagnostic Pathology  
**Keyword:** Bone and Joint

HEMOPHILIC POLYARTHROPATHY IN A DOG: CLINICO-PATHOLOGIC FINDINGS

S.F. Santagostino¹, J.B. Engiles², B.J. Turek³, and M.D. Sánchez⁴  
¹Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, ²Department of Pathobiology - New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

A 2-month-old intact male Great Dane presented for ambulatory difficulties with severe swelling and pain of the right and left stifle joints and right tarsus. Radiographs revealed soft tissue swelling, widening of articular spaces, osteopenia, and enlargement of the distal epiphyses. Complete blood count and coagulation tests indicated regenerative anemia, thrombocytopenia, prolonged PTT and severe reduction of factor VIII levels. Autopsy revealed widespread subcutaneous, intramuscular and periarticular ecchymoses with edema and hemarthrosis. Synovial fluid had reduced viscosity with thickening and hyperemia of the synovial membranes. There was erosion of the articular cartilage with exposure of subchondral bone and osteophytes on the right humeral condyles, the right olecranon fossa, and the distal femoral condyles. Histology revealed the presence of abundant intraarticular granulation tissue with pannus formation, hemorrhage, and a diffuse lymphoplasmacytic and proliferative synovitis with edema and periarticular osteophytes. These lesions were compatible with a subacute hemophilic arthropathy which describes degenerative joint lesions following recurrent hemarthrosis secondary to factor VIII deficiency. In addition, growth plates contained irregular cartilaginous blebs and islands of disorganized matrix with dysplastic chondrocytes, multiple linear fissures and aberrant blood vessels. Hemophilic arthropathy is a rarely described manifestation of factor VIII deficiency in dogs. Although the pathogenesis is not fully elucidated, the excessive iron deposition from repeat intraarticular bleeding may trigger inflammation with damage to the synovium and articular cartilage, synovitis and pannus formation. The findings of hemarthrosis, joint swelling and recurrent shifting lameness in juvenile dogs may represent important clinical indicators of hemophilia.

**Poster Number:** D-20

**Section:** Diagnostic Pathology  
**Keyword:** Alimentary
HISTOLOGIC CHARACTERIZATION OF THE CANINE ESOPHAGUS AND
CORRELATIONS WITH PRIMARY AND SECONDARY ESOPHAGEAL
DISEASES

M.A. McCarthy, P. Pesavento, and S. Marks
School of Veterinary Medicine, University of California – Davis, Davis, CA

Complete microscopic characterization of the normal canine esophagus is lacking, and careful histologic
assessment of esophageal microanatomy is critical to interpret the pathophysiology of both primary and
secondary esophageal disease. In particular, given the relative accessibility of the esophagus for
endoscopic biopsy, correlation of biopsy with the spectrum of normal histology of the esophagus at a
given level is crucial for interpretation. Further, characterization is needed before interpretation of
primary esophageal diseases (e.g. megaesophagus, reflux, and dysplasia), and to understand
correlations between gastrointestinal disease and histologic esophageal lesions, which are currently not
established for any veterinary species. Finally, the potential utility of esophageal biopsies or necropsy
samples in disease diagnosis is largely unexplored. This study examines 20 clinically normal canine
esophagi cataloging epithelial, glandular, muscular, and nerve components along the entire esophageal
length. This examination provides a guide to correct sampling and interpretation of canine esophageal
microanatomy. To investigate potential correlation of histologic features with primary and secondary
esophageal diseases, a collection of retrospective cases of megaesophagus, esophagitis, leiomyositis,
and intestinal lymphoma from the archives of the UC Davis Veterinary Medical Teaching Hospital are
examined.

Poster Number: D-21
Section: Diagnostic Pathology Focused Group Session II
Keyword: Alimentary

HISTOPATHOLOGIC CHARACTERIZATION OF HOLSTEIN CATTLE
NATURALLY INFECTED WITH SALMONELLA DUBLIN

H.L. Pecoraro\textsuperscript{1}, G.E. Duhamel\textsuperscript{1}, and B. Thompson \textsuperscript{2}
\textsuperscript{1}Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,
\textsuperscript{2}Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY

\textit{Salmonella enterica} subsp. \textit{enterica} serovar Dublin (\textit{Salmonella} Dublin) is a cattle-adapted bacterium,
causing high morbidity in dairy cows. Clinical signs range from asymptomatic to diarrhea to systemic
infection. Previous research has done much to elucidate the pathogenesis and describe histologic
changes during \textit{Salmonella} Dublin infection; however, these studies have been done in experimentally-
infected animals. The object of the current study is to characterize common histopathologic lesions of
cows naturally-infected with \textit{Salmonella} Dublin. To this end, a retrospective search of the archives at
Cornell University College of Veterinary Medicine Animal Health Diagnostic Center was performed,
yielding 51 cases of culture-confirmed \textit{Salmonella} Dublin with corresponding tissue histology. Of the 51
cases, 51 were Holstein cows, 48 were female, 44 were under 6 months of age, and 47 were from NY or
PA. There were 28 bacterial, 8 viral, and 3 parasitic co-infections. Mild to severe myocarditis was found
in over 70% (8/11) of heart tissues. Eighty-six percent (44/51) of lung tissues were characterized by moderate to severe pneumonia. In addition, moderate to severe inflammation was observed in 55% (19/34) of liver, 50% (10/20) of splenic, and 40% (14/35) of lymph node tissues. Inflammation was primarily composed of neutrophils, with fewer lymphocytes and histiocytes, and was often accompanied by necrosis. Based on the histopathology, we propose a case definition of *Salmonella* Dublin in young (<6 month old) Holsteins, which will, ultimately, assist in the development of improved protocols for the diagnosis of infectious diseases of dairy cattle.

**Poster Number:** D-22

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Neoplasia

**HYPERTROPHIC OSTEOPATHY IN A DOG WITH DISSEMINATED HISTIOCYTIC SARCOMA**

S. Choudhary¹, G. Andrews¹, D. S. Biller², S. Hocker², and J. Ryseff¹

¹Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; ²Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Hypertrophic osteopathy (HO) is a rare disease of undetermined pathogenesis reported most commonly in humans and dogs. HO is characterized by the deposition of periosteal new bone, and commonly occurs due to neoplastic or infectious masses in the thoracic or less often abdominal cavities. A 3-year old, intact female Bernese mountain dog was evaluated for lameness and fever of 3 weeks duration. Radiography revealed periosteal bony proliferation on the proximal diaphysis of the 3rd, 4th, 5th, and 8th ribs and lateral margins of the wing of the left ilium. Blood changes included mild normochromic normocytic nonregenerative anemia, mild thrombocytopenia, and elevated creatine kinase. Ultrasound examination revealed the presence of multiple masses in the spleen that cytologically consisted of sheets of histiocytic cells with hemophagocytosis. Due to a poor prognosis the dog was euthanized. At necropsy, the head, neck, and tubercle of the left 3rd, 4th, 5th, and 8th ribs and the cranial dorsal surface of the left wing of the ilium had rough, mildly thickened periosteal surfaces. Microscopically there was periosteal proliferation consisting of bony and fibrous tissue. The spleen, lungs, liver, kidneys and pancreatic lymph node contained multiple coalescing, white to tan, raised nodules. These nodules consisted of neoplastic histiocytic cells, which were also detected microscopically in the bone marrow and adrenal glands. A diagnosis of histiocytic sarcoma and HO was made based on the clinical, pathologic, and immunohistochemical findings. To the author’s knowledge, this is the first published report of HO secondary to disseminated histiocytic sarcoma.

**Poster Number:** D-23

**Section:** Diagnostic Pathology

**Keyword:** Liver and Pancreas

**IMMUNOHISTOCHEMICAL CHARACTERIZATION OF COMBINED HEPATOCELLULAR AND CHOLANGIOCELLULAR CARCINOMA IN THE DOG**
Combined hepatocellular and cholangiocellular carcinomas (cHCC-ChC) are rare hepatic tumors, representing <1% of all primary liver tumors in human beings. Phenotypically they are a heterogeneous group of tumors, but characteristically contain intimately associated neoplastic hepatocytes and biliary epithelium. Recently hepatic progenitor cells have been implicated as the cell of origin in human beings. Search of the Cornell University Anatomic Pathology database identified three dogs diagnosed with cHCC-ChC between 2004 and 2014. Histologic examination confirmed uni- or multinodular hepatic masses composed of neoplastic hepatocytes intermixed with islands of neoplastic biliary epithelium. In each case the predominant morphology of hepatocellular components was a well-differentiated, trabecular phenotype. In all cases the cholangiocellular component was moderately to well-differentiated, and in two cases was accompanied by a dense desmoplastic response. Distribution of the cholangiocellular carcinoma was either as distinct islands (2/3) or was diffusely intermixed (1/3) with neoplastic hepatocytes. In one case a distinct intermediate cellular morphology was evident in the transitional zone. Immunohistochemistry for CK19 and HepPar-1 confirmed hepatocyte and biliary epithelial differentiation. Biliary epithelium showed variable SOX2 immunoreactivity, with strongest reactivity observed in the transitional zone in two cases. Neoplastic biliary epithelium displayed variable vimentin immunoreactivity in two dogs. These cases describe the immunophenotypic diversity of CHCC-ChC in the dog and to the author’s knowledge is the first report of stem cell immunoreactivity in this rare canine tumor.

Poster Number: D-24

Section: Diagnostic Pathology
Keyword: Infectious Disease

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE GLOBAL ISCHEMIC-RELATED CHANGES AND THE ROLE OF APOPTOSIS IN THE BRAIN OF CATS INFECTED WITH CYTAUXZOOON FELIS

L. Clarke and D.R. Rissi
Department of Pathology and Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA

We have recently described in detail the neuropathological changes related to vascular occlusion and ischemia caused by *Cytauxzoon felis* infection in cats. In addition to the multifocal ischemic-related changes, we have subjectively detected widespread glial changes that could account for a global reaction to hypoxia/ischemia. We also hypothesize a potential role of apoptosis leading to cell death. To confirm our first hypothesis, sections of brain from 8 affected cats and 8 age-matched control cats were submitted to immunohistochemistry for GFAP and CD18. Immunostaining was evaluated using Image-Pro Plus™ software on 10 random high-power fields (HPF at 400 x) from the gray matter and 10 random HPF from the white matter. The number of positive astrocytes and microglia, as well as the average
astrocytic cytoplasmic area, were quantified, and a mean value for the gray and white matter in both groups was generated. Widespread astrocytic hypertrophy and hyperplasia with microglial hyperplasia were detected in all affected cats, confirming a global reaction to the underlying hypoxic/ischemic insult due to vascular occlusion by schizont-laden macrophages. Immunostaining for caspase-3 was detected in intravascular and perivascular macrophages in the leptomeninges and less often gray and white matter in all affected cats. Cats with encephalomalacia (4 cases) had additional cytoplasmic immunostaining of microglial cells/macrophages around the necrotic foci and macrophages and cell debris within the areas of necrosis. These results indicate a global reaction of brain tissue to hypoxia/ischemia and a potential role of apoptosis in the pathogenesis of *C. felis* infection in cats.

**Poster Number:** D-25

**Section:** Diagnostic Pathology  
**Keyword:** Respiratory System

**INFLTRATIVE EXTRAMEDULLARY PLASMACYTOMA OF THE RESPIRATORY TRACT WITH LYMPH NODE METASTASIS AND INTRAHISTIOCYTIC AMYLOID**

S. Sykes¹, V. Byfield¹, L. Sullivan³, S. Bender¹, P.F. Moore⁴, and M.D. Sánchez¹  
¹University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, ²Red Bank Veterinary Hospital, Hillsborough, NJ, ³Murdoch University School of Veterinary and Life Sciences, Perth, Australia, ⁴University of California School of Veterinary Medicine, Davis, CA

An infiltrative extramedullary plasmacytoma (EMP) of the respiratory tract with lymph node metastasis in a domestic longhaired cat is described. The patient presented for nasal discharge, mandibular lymphadenopathy and anorexia which progressed to respiratory distress. Biopsies of the nasal turbinates and frontal sinus revealed marked plasmacytic infiltrates; differential diagnoses included severe plasmacytic inflammation and EMP. An additional biopsy of the mandibular lymph node revealed plasmacytic and histiocytic infiltrates with abundant intrahistiocytic and lesser extracellular congophilic material. Autopsy examination revealed thickened nasal turbinates and soft palate, and friable pink-tan material within the frontal sinuses, nasal cavity, and nasopharynx. The lungs were slightly wet with multifocal irregular tan 0.2 – 1 cm diameter friable nodules. Multiple lymph nodes were enlarged, friable and red-tan with loss of architecture bilaterally. Histopathology revealed a mature-type EMP within the frontal sinuses and respiratory tract, including the nasal cavity, larynx, trachea and lungs. Admixed with the neoplastic cells within the lymph nodes was marked granulomatous inflammation with extensive intrahistiocytic (and lesser extracellular) amyloid. Neoplastic cells were CD79a and MUM1 positive, and PARR revealed a monoclonal rearrangement of the KDE to a Kappa Variable segment (KDev). Immunohistochemistry of the amyloid was moderately positive for lambda light chain, and negative for kappa light chain and AA amyloid. Electron microscopy confirmed abundant intrahistiocytic amyloid. EMPs are rarely reported in cats, and are typically cutaneous neoplasms. Although primary local amyloidosis with secondary granulomatous inflammation is reported in plasma cell dyscrasias in animals, extensive accumulation of intrahistiocytic amyloid is unusual.

**Poster Number:** D-26
INTRACOELOMIC NEOPLASIA IN A CROWNTAIL SIAMESE FIGHTING FISH (BETTA SPLENDENS)

K. Eden¹, J.F. Edwards², D.P. Sponenberg³, and B.F. Porter²
¹Department of Biomedical and Veterinary Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, ²Texas A&M University College of Veterinary Medicine and Biomedical Science, College Station TX, ³Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA

A two-year-old, tricolor, male crowntail betta (Betta splendens) developed coelomic distention over the course of three months. Coelomic tap revealed 0.5cc of acellular, light yellow fluid. The fish eventually became anorexic and unable to swim properly, and was humanely euthanized. A 1cm in diameter, tan, firm, well-demarcated, irregular mass was within coelomic cavity, extending from the dorsal operculum to the kidneys, displacing major organs. Histologically, the mass was composed of cords, tubules, and papillary projections of compact, basophilic, columnar epithelial cells which often piled in pseudostratified patterns and occasionally displayed faint cilia. Deposits of fibrin, proteinaceous fluid, preexisting stroma, infiltrating mononuclear cells, and necrosis interdigitated with the neoplasm. A diagnosis of epithelial neoplasia, likely originating from the respiratory epithelium of the labyrinth organ or cranial swim bladder, was made based on morphology. Other epithelial differentials including renal, thyroid, and intestinal carcinomas could not be completely ruled out; immunohistochemistry was unable to further confirm cell of origin. This case represents the inherent challenges in ornamental fish pathology due to several factors including the paucity of reports on naturally-occurring neoplasia as compared to that of mammalian pets. Neoplasia in bettas has not been a well characterized despite their popularity in the pet industry and national/international aquatic animal show circuits. Investigation of these lesions would be of interest to many hobby and show breeders who are particularly concerned with stock genetics.

Poster Number: D-27

LIVER AND SPLEEN LIGHT CHAIN DEPOSITION DISEASE IN A DOG

H.L. Pecoraro¹, S.P. McDonough¹, S.A. Center², S.M. Liu³, and K. Gisselman⁴
¹Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, ²Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, ³IDEXX Veterinary Services, Memphis, TN, ⁴Hope Advanced Veterinary Center, Vienna, VA

Monoclonal plasma cell proliferative disorders include diseases that deposit organized fibrillar material, such as amyloidosis, and diseases that form non-organized, electron-dense granular deposits, such as light and heavy chain deposition diseases. While light chain amyloid deposition has been observed in dogs and cats with extramedullary plasmacytomatas, there are no published reports of light chain
deposition disease in domestic animals. Recently, a 13 year old, male neutered West Highland White Terrier dog with a history of chronically elevated alkaline phosphatase, hyperglobulinemia, hypoalbuminemia, and treatment with d-Penicillamine for a severe copper-associated hepatopathy presented for chronic vomiting, proteinuria, and suspected pancreatitis. Imaging showed hepatomegaly, as well as a splenic mass. On histopathology, an amorphous, non-fibrillar, eosinophilic material lined the spaces of Disse and aggregated within the red and white pulp regions of the liver and spleen, respectively. Histochemical and immunohistochemical stains revealed the material was non-congophilic, trichrome chromophilic, PAS-positive diastase-resistant, and had moderately strong immunoreactivity to kappa light chain antibodies, findings consistent with kappa light chain deposition. To our knowledge, this is the first report of light chain deposition disease in the dog and represents an uncommon manifestation of monoclonal plasma cell proliferative disorder in this canine patient.

**Poster Number:** D-28

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

### MAMMARY RHABDOMYOSARCOMA WITH WIDESPREAD METASTASIS IN AN ADULT FEMALE DOG

L. Dagher, A. G. Armien, J. Dundas, and M.M. Dennis

A ten year old female, mixed breed dog, presented for acute dyspnea developing nine months after multiple mammary tumors were identified. Necropsy revealed widespread embryonal rhabdomyosarcoma, involving the largest mammary tumor, spleen, kidney, liver, lung, and heart. Microscopically, neoplasms comprised a mixture of myoblastic and myotubular cells, the latter occasionally showing rudimentary sarcoplasmic cross-striations on H.E. stained preparations. These primitive sarcoplasmic cross-striations were enhanced by polarized light on preparations stained with Picosirius red stain. Immunohistochemically, tumor cells were strongly positive for vimentin and desmin and were negative for myoglobin and smooth muscle actin. Electron microscopy revealed cytoplasmic myofilaaments with early sarcomere differentiation and z-band like structures. Rhabdomyosarcoma is a rare neoplasm of dogs and this case is especially unusual due to the apparent origin in mammary gland or associated skin and the old age of the dog.

**Poster Number:** D-29

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

### METASTATIC THYMOMA IN A DOG

K.L Hughes, V. Wiles, N.Leibman, and A.C. Avery

A 12 year-old, neutered, male, Portuguese water dog presented for a 4 month history of a mediastinal mass and hepatosplenomegaly with a year history of continuous hyporexia and diarrhea. Abdominal ultrasound showed nodular hepatopathy, diffuse enteropathy, and diffuse splenomegaly. Fine needle
aspirates of liver, spleen and mediastinal mass were suspicious for lymphoma but not diagnostic. Flow cytometry was performed separately for the mediastinal mass and combined liver/spleen. The flow cytometry studies revealed an expansion of small CD4+CD8+ lymphocytes at both sites. PCR for Antigen Receptor Rearrangement (PARR) revealed a polyclonal amplification of T cell receptor genes and no amplification of immunoglobulin genes. A biopsy of the mediastinal mass consisted of sheets and aggregates of neoplastic polygonal cells with heterogeneous scattered aggregates of lymphocytes consistent with thymoma. Also on biopsy, over 90% of the liver was effaced and replaced by sheets of similar polygonal neoplastic cells as seen in the thymus with scattered heterogeneous lymphocytes with few remaining atrophied hepatic cords. Identification by flow cytometry of T cells with co-expression of CD4 and CD8 on the mediastial mass is diagnostic for thymoma, however, before T cells leave the neoplastic thymus, they down-regulate either CD4 or CD8 and CD4+CD8+ T cells are not seen in the periphery. Finding CD4+CD8+ T cells in the spleen/liver sample on flow cytometry suggested either a metastatic thymoma with ectopic T cell development or a very rare CD4+CD8+ lymphoma. The biopsy confirmed metastatic thymoma, which was consistent with flow cytometry and PARR results.

**Poster Number:** D-30

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

**MICROSPORIDIOSIS IN A BEARDED DRAGON (POGONA VITTICEPS)**

K. Sakaguchi, Y. Sokolova, C. Higbie, J. Nevarez, and D. Paulsen

A 10-month-old, female bearded dragon was submitted for necropsy. The postmortem examination revealed a 35 x 25 x 20 mm, tan to red, soft cardiac mass, which had multifocal to coalescing, yellow to red, slightly raised foci, up to 5 mm in diameter, on its surface and contained large amounts of red to tan gelatinous and friable material. The right tarsal joint was replaced by a soft white mass measuring 10 x 4 x 3 mm. Microscopically the cardiac and joint lesions were characterized by granulomatous inflammation with intralesional organisms. There were bilateral paraovarian granulomas with larger numbers of similar organisms. Smears prepared from formalin-fixed tissues stained with Trichrome Blue and Calcoflour White demonstrated characteristic staining for microsporidian spores, which were oval and 2.1 +/- 0.07 μm x 1.1 +/- 0.07 μm (n=11) in size. Transmission electron microscopic examination revealed uninucleate spores exhibiting a relatively thick endospore, an undulating exospor, and two to five pairs of polar filament coils. Many spores resided in parasitophorous vacuoles. PCR with specific microsporidian primers amplified SSUrDNA from DNA isolated from the cardiac granuloma, and its sequence was 98% identical to Encephalitozoon cuniculi. The genus Encephalitozoon is ubiquitous and infects a variety of hosts from insects to humans. However, microsporidiosis in reptiles is extremely rare, and there are only two reports of systemic microsporidiosis in bearded dragons. In the most recent report infection was by an unknown genotype of E. cuniculi. Further molecular characterization is ongoing to identify the genotype in the present case.

**Poster Number:** D-31

**Section:** Diagnostic Pathology  
**Keyword:** Nervous System
NEUROLOGIC AMEBIASIS CAUSED BY BALAMUTHIA MANDRILLARIS IN AN INDIAN FLYING FOX (PTEROPUS GIGANTEUS)

N. Crossland¹, I.K. Ali ⁴, C.T. Higbie³, J.W. Jackson ⁴, G. Pirie ⁵, R.W. Bauer¹,²
¹Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA, ²Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, Baton Rouge, LA, USA, ³Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA, ⁴National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA, ⁵Baton Rouge Zoo, Baton Rouge, LA, USA

A 5 month old intact male Indian flying fox (Pteropus giganteus) that presented with an acute onset of obtundation was diagnosed with amebic encephalitis. Histopathologic examination revealed numerous amebic trophozoites within necrotic foci, affecting the occipital cerebrum and surrounding the mesencephalic aqueduct. The etiologic agent, Balamuthia mandrillaris, was determined by multiplex quantitative polymerase chain reaction (qPCR), immunohistochemistry (IHC), and indirect immunofluorescence (IIF) assay. To the authors’ knowledge this is the first documented case of amebic encephalitis within the order Chiroptera.

**Poster Number:** D-32

**Section:** Diagnostic Pathology Focused Group Session II

**Keyword:** Wildlife

OLFACTORY NEUROBLASTOMA IN A FLORIDA BLACK BEAR (URSUS AMERICANUS FLORIDANUS)

G. Krane¹, A. Miller³, C. Cummings³, D. Wolf⁴, and S. Craft¹
¹University of Florida College of Veterinary Medicine, Gainesville, FL, ²Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, Section of Anatomic Pathology, Ithaca, NY, ³Ultrapath Imaging; Durham, NC, ⁴Florida Fish and Wildlife Conservation Mission

Neoplasia affecting the central nervous system is uncommonly reported in members of the Ursidae family. An adult male, Florida black bear (Ursus americanus floridanus) was euthanized due to increased human interactions. Necropsy revealed multifocal, soft, red, friable masses in the frontal sinus, nasopharynx, and oral cavity that invaded the cribiform plate and the olfactory lobe.

Histopathology revealed a densely cellular, unencapsulated mass of anastomosing islands of round to polygonal cells supported by a fine arborizing fibrovascular stroma. Cells had scant cytoplasm and round to ovoid nuclei. Moderate anisocytosis and anisokaryosis were present, and mitoses were ~10/400x field. Cells occasionally formed Flexner-Wintersteiner rosettes and less frequent perivascular pseudorosettes. The neoplastic cells lacked immunoreactivity for CD3, CD79a, pancytokeratin, GFAP, NSE, synaptophysin, and neurofilament. Based on histologic features, the mass was diagnosed as an olfactory neuroblastoma. Ultrastructural analysis is pending.

Olfactory neuroblastoma (esthesioneuroblastoma) is a very rare tumor of olfactory neuroepithelium. In
cats and dogs, they grossly behave similar to the mass in this bear. Microscopically, islands of cells with an arborizing fibrovascular stroma form pseudorosettes and true rosettes, with Flexner-Wintersteiner more prevalent than Homer-Wright.

Olfactory neuroblastoma has been reported in a variety of species including dog, cat, horse, cow, and laboratory mice. To our knowledge, this is the first report of this tumor in Ursidae, making a unique contribution to the scientific literature.

Poster Number: D-33

Section: Diagnostic Pathology
Keyword: Nervous System

OLIGODENDROGLIOMA WITH SPINAL METASTASIS IN DOGS: AN UNDERREPORTED NEOPLASM?

T. Huynh, M.J. Cruz Penn, and A.B. Rogers
Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA; Smithsonian National Zoological Park, Washington, DC

Oligodendrogliomas are common neoplasms in mature dogs that generally present as a solitary intracranial mass. Atypical manifestations of this neoplasm with cranial and spinal involvement are associated with spread via the ventricular system and/or leptomeninges. We report two cases of multifocal oligodendrogliomas presenting within a short time frame. CASE 1: A 9-year-old, neutered male Boston terrier with 1 week history of forelimb paresis. MRI revealed contrast-enhancing lesions within the forebrain, central canal, and meninges of the cervicothoracic spinal cord. Grossly, an indistinct tan mass protruded into the left lateral ventricle. Histologically, neoplastic cells consistent with oligodendroglioma were found within the left lateral and fourth ventricles, central canal and leptomeninges of the cervical spinal cord, and left forebrain. CASE 2: A 7-year-old, neutered male Boxer with 1.5 week history of tetra-ataxia and seizures. MRI was not performed. Grossly, a gray-tan, gelatinous mass occluded the left lateral ventricle and similar 2 mm mass was attached to the dura at the foramen magnum. Histology was consistent with oligodendroglioma. There was minimal periventricular infiltration with extension into the fourth ventricle and subarachnoid spaces of the caudoventral cerebrum and entire spinal tract. Immunohistochemistry was performed to further support a diagnosis of oligodendroglioma in both cases. Our findings confirm and extend recent reports of oligodendrogliomas manifesting as primary intraventricular and/or multicentric neoplasms (DR Rissi et al, JVDI, 2015; MW Koch et al, JAAHA, 2011) suggesting that routine examination of the brain and spinal cord should be performed to determine the full extent of canine oligodendrogliomal invasion.

Poster Number: D-34

Section: Diagnostic Pathology
Keyword: Neoplasia

PATHOLOGICAL STUDY ON PRIMARY LEPTOMENINGEAL HISTIOCYTIC SARCOMA IN DOGS
A. Thongtharb, K. Uchida, J.K. Chambers, and H. Nakayama  
Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan

Histiocytic sarcoma (HS) is a progressive and fatal malignant neoplasm that occurs mainly in middle- to old-aged dogs. This study describes histological and immunohistochemical characteristics of leptomeningeal HS and immunocytotoxicological features of 2 newly established HS cell lines. Twenty-one canine brain tumors, 14 males and 7 females with the average of 9 years old, were evaluated. The MRI confirmed that most of the tumors were located in the cerebrum. However, cerebellar tumors were noted in 2 dogs. Microscopically, the tumors were classified into 2 histological patterns determined mainly by the tumor cell morphology; group 1 - round to polygonal and group 2 - spindle shaped. The tumors in group 1 had a higher mitotic rate and a higher hemophagocytic ability compared to those in group 2. Tumor cells of all the 21 tumors were positive for HLA-DR, Iba-1 and CD204, those of 19 for iNOS, those of 17 for CD163, those of 8 for lysozyme and CD208, and those of 5 for S100. However, no association was found between the histological patterns and the expression of the markers. In addition, a newly established cell line from leptomeningeal HS was positive for all markers mentioned above, whereas the other HS cell line obtained from the skin expressed only dendritic cell phenotypes (CD1+, CD54+ and CD68). These results suggest that canine primary leptomeningeal HS tends to exhibit both phenotypes of histiocytic differentiation (macrophages and dendritic cells).

Poster Number: D-35

Section: Diagnostic Pathology  
Keyword: Bone and Joint

PIGMENTED VILLONODULAR TENOSYNOVITIS IN A RETICULATED GIRAFFE (GIRAFFE CAMELOPARDALIS RETICULATA)

E.A. Ihms, A. Rivas, E. Bronson, and L.M. Mangus  
Johns Hopkins University Department of Molecular and Comparative Pathobiology; The Maryland Zoo in Baltimore

An adult female reticulated giraffe was euthanized due to chronic, progressive forelimb lameness that was localized to the right fetlock and refractory to therapy. Necropsy and histopathology were performed, resulting in a diagnosis of pigmented villonodular tenosynovitis (PVNS), a condition commonly occurring in the extremities of human patients but rarely reported in veterinary species. PVNS is characterized by one or more encapsulated tan pigmented nodules within the synovium and/or tendon sheath of the distal extremities. Nodules are characterized histologically by abundant connective tissue, heavy macrophage and fibroblast infiltration, and lower numbers of large multinucleated giant cells which morphologically resemble osteoclasts. The pathophysiology of PVNS is poorly understood, and there is debate concerning whether this lesion represents a primarily neoplastic or inflammatory process. In the case of this giraffe, immunohistochemical analysis was performed on fixed tissues to assess to expression of the macrophage markers lba-1 and lysozyme, as well the cysteine protease cathepsin K, which is typically considered an osteoclast marker, but has also been demonstrated in conditions resulting in high levels of macrophage activation. Multinucleated cells showed moderate cell membrane-associated immunoreactivity for lba-1, strong diffuse cytoplasmic reactivity for cathepsin K,
and were negative for lysozyme. These IHC findings suggest the large multinucleated cells characteristic of PVNS are of monocyte-macrophage origin, consistent with findings in human patients. This case report is the first documented case of PVNS in a non-domestic ungulate, and provides further histomorphologic and immunohistochemical characterization of an unusual condition which affects both human and animal patients.

Poster Number: D-36

Section: Diagnostic Pathology Focused Group Session I
Keyword: Nervous System

PRIMARY LEPTOMENINGEAL GLIOMATOSIS IN A DOMESTIC SHORTHAIR CAT

W.M. Zoll¹, J.M. Rudnick¹, T. Keeshen¹, C. Bandt¹, A.D. Miller², and J.R. Abbott¹
¹College of Veterinary Medicine, University of Florida, Gainesville, FL; ²Section of Anatomic Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY

Gliial tumors are uncommon in cats compared to their canine counterparts. A 15-year-old neutered male domestic shorthair cat presented to the University of Florida Veterinary Hospital with a 16-day history of hindlimb paralysis in conjunction with a 1-week duration of inappetence and lethargy. The cat was obtundated, had a temperature of 95.9°F, systolic blood pressure of 100, and 6% dehydration. Bloodwork was unremarkable and IV fluids were given. The patient had multiple seizures and euthanasia was elected. The most significant finding at gross examination was multifocal, mild protrusions of discs into the thoracic and lumbar spinal canal. Associated intervertebral disk spaces contained fragmented, tan material consistent with intervertebral disc disease. Histologic examination of cervical, thoracic and lumbar spinal cord and the myelencephalon revealed a moderately cellular, well demarcated neoplasm predominantly confined to the subarachnoid space composed of small round to polygonal cells forming packets and clusters interspersed with scant stroma. Neoplastic cells ranged from round to spindloid with a scant amount of cytoplasm and low mitotic rate. Diffusely, the neoplastic cells had strong intranuclear immunoreactivity for Olig2. The supporting stroma stained positive with anti-glial fibrillary acidic protein (GFAP) antibody. Interspersed throughout the neoplasm were scattered CD3+ lymphocytes and Iba-1+ macrophages. No immunoreactivity for CD45 and PAX5 was detected. Based on the histologic and immunohistochemical findings, this case represents the first known report of diffuse leptomeningeal gliomatosis in a cat. With no primary mass in this animal, it is presumed that the tumor arose from ectopic glial rests within the meninges.

Poster Number: D-37

Section: Diagnostic Pathology
Keyword: Neoplasia

PRIMARY MALIGNANT HISTIOCYTOSIS IN THE BRAIN OF AN ITALIAN GREYHOUND

N. Pate¹, A. Roland², L. Gainsburg³, D.L. Kraitchman¹, P.R. Gavin⁵, and J. Mankowski¹
A 12-year-old, male neutered, Italian Greyhound with a one-week history of anxiety, excessive vocalization, and intermittent anorexia was referred for neurologic evaluation. On MRI, multifocal contrast-enhancing lesions were present affecting the falx cerebri rostral to the lateral ventricles and the ventrolateral right cerebrum adjacent to meninges. Histopathologically, the grey matter of the frontal lobe and overlying meninges were focally disrupted by a poorly demarcated, invasive and expansive mass composed of pleomorphic neoplastic round cells with a high mitotic rate. Neoplastic cells had several features suggestive of histiocytic origin including abundant cytoplasm, and occasional erythrophagcytosis. Multinucleated giant cells were also present. By immunostaining, infiltrating cells were positive for Iba-1 and CD136 and negative for kappa and lambda light chain, CD3, CD20, and GFAP. Cellular morphology and immunohistochemical profile were consistent with primary malignant histiocytosis. Diagnosis of histiocytic tumors can be challenging with histopathology alone, and the use of immunohistochemistry can be advantageous. In general, malignant histiocytic tumors exhibit positive staining with a variety of markers including CD1c, CD11c, CD45, lysozyme, CD18, and Iba1, and do not immunostain for B cell, T cell, and glial cell markers. Primary histiocytic tumors involving the brain are rare with an overall poor to grave prognosis due to their invasive and aggressive nature.

**Poster Number:** D-38

**Section:** Diagnostic Pathology

**Keyword:** Rabbit

**PULMONARY CARCINOSARCOMA IN A RABBIT**

S. Choudhary, A. Kumar, and G. Andrews
Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Pulmonary carcinosarcoma is exceedingly rare and highly malignant neoplasm in animals and human beings with a poor prognosis. It is characterized by a biphasic histopathological pattern consisting of both carcinomatous and sarcomatous components. An 8-year old, intact male European rabbit was received for necropsy with history of intermittent anorexia of 2 weeks duration. On clinical presentation, he was severely underweight and had increased respiratory effort and mild ocular and nasal discharge. Physical examination revealed a mass on the right thorax and potentially another mass behind the right kidney. Serum chemistry changes included significantly elevated levels of alanine transaminase, alkaline phosphatase, and blood urea nitrogen. The rabbit died suddenly 24 h after initial examination. At necropsy, the right rib cage contained a large, grey-white to tan, irregular, firm mass involving the bodies and intercostal spaces between the 7th and 9th ribs. There were numerous greyish-white firm nodules 0.5-5 cm in diameter scattered throughout all lung lobes. Small numbers of similar nodules were present in the liver and the kidneys. Microscopically, the nodules in the lungs were composed of an adenocarcinomatous component with acinar-like structures irregularly intermingled with sarcomatous neoplastic cells with bone and rare cartilage differentiation. The mass on the right rib
cage and nodules in the liver and kidneys were composed of a sarcomatous element. A diagnosis of pulmonary carcinosarcoma was made based on the characteristic histopathologic and immunohistochemical findings. To the author’s knowledge, this is the first published report of malignant pulmonary carcinosarcoma in a rabbit.

**Poster Number:** D-39

**Section:** Diagnostic Pathology Focused Group Session I
**Keyword:** Respiratory System

**SPONTANEOUS PULMONARY HYALINOSIS IN THREE SUGAR GLIDERS (PETRAURUS BREVICEPS)**

S.A. Sokol, T.L. Southard, and A.D. Miller
Section of Anatomic Pathology, Department of Biomedical Science, College of Veterinary Medicine, Cornell University, Ithaca, NY

Pulmonary hyalinosis is an idiopathic, typically incidental lesion of old dogs, characterized by multifocal aggregates of epithelioid and multinucleate macrophages that surround periodic acid-Schiff (PAS)-positive hyaline material in airways. Pulmonary hyalinosis is considered a type of alveolar filling disorder, a group of conditions defined by the accumulation of abnormal material within alveoli that also includes alveolar histiocytosis, endogenous lipid pneumonia, alveolar proteinosis, alveolar phospholipidosis, and alveolar microlithiasis. Lung lesions resembling pulmonary hyalinosis were observed in three adult sugar gliders (2 females and 1 male). Clinical signs for two of the sugar gliders included lethargy, tachypnea, and dyspnea, and one was dead-on-arrival with no clinical history; but at necropsy, all three animals had other significant lesions to explain the cause of death or clinical signs prior to euthanasia. Gross lesions were characterized by mildly firm, discolored, vaguely nodular areas of pulmonary parenchyma. Histologic examination of the lung revealed granulomatous inflammation with intracellular amphophilic hyaline bodies within alveoli and bronchioles. Hyaline bodies were positive for PAS, crystal violet, oil red O, and displayed birefringence under polarized light, similar to findings in dogs with pulmonary hyalinosis. To the authors’ knowledge, this is the first report of spontaneous pulmonary hyalinosis in sugar gliders or in any non-canine species.

**Poster Number:** D-40

**Section:** Diagnostic Pathology
**Keyword:** Neoplasia

**SUBSEQUENT UNRELATED CUTANEOUS T-CELL LYMPHOMA IN A DOG WITH PREVIOUSLY DIAGNOSED T-ZONE LYMPHOMA**

F. Brooks¹, K. Santangelo², A.C. Avery², and A.R. Moore²
¹TDDS, Exeter, UK, ²Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, USA
**Case Report:** 12yr, German Shepherd mix dog presented with an enlarged right axillary lymph node and peripheral lymphocytosis. Lymph node cytology had an increase in medium lymphocytes; and was T-cell PARR positive. Flow cytometry on the blood revealed a CD4+CD45- lymphocytosis; identical clonally rearranged T cell receptors were present in blood and lymph node suggesting a T-zone lymphoma with lymphocytosis. Eight months later, the T-zone lymphoma was in remission following treatment when this patient presented with a subcutaneous mass. FNAs of the mass found cells that do not have a classical T-Zone lymphoma morphology and were more suggestive of a histiocytoma. Given the clinical history, further investigation was recommended. Multiplex Immunocytochemistry identified the cells as CD3+Vimentin+CD79a-Cytokeratin-CD18-; consistent with a T-cell neoplasm and excluding histiocytoma. Because of the visual difference from T-zone lymphoma, flow cytometry was performed and revealed a CD4-CD5-CD8+CD3+CD45+ lymphocytosis, which was immunophenotypically distinct from the previous T-zone population and supported the diagnosis of cutaneous T-cell lymphoma. PARR analysis demonstrated a different monoclonal rearrangement between the T-zone lymphoma and cutaneous lymphoma.

**Discussion:** Cytology of the mass did not match the original T-zone lymphoma and was misleading, suggesting a round cell neoplasm other than the true diagnosis. The use of ancillary diagnostics allowed accurate identification and distinction of two separate neoplasms without reliance on more invasive techniques. This case highlights challenges of diagnosing round cell neoplasms and suggests that ancillary testing may be appropriate and beneficial in ambiguous cases.

**Poster Number:** D-41

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Avian

**SUSPECTED FENBENDAZOLE TOXICITY IN AN AMERICAN WHITE PELICAN (PELECANUS ERYTHROHRYNCHOS)**

I.J. Kim¹, J.C. Nietfeld¹, D.M. Lindemann², and D. Eshar²

¹Kansas State Veterinary Diagnostic Laboratory, Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, ²Departments of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS

A wild-raised, 5.0 kg, male American white pelican (Pelecanus erythrorhynchos) of unknown age had a decreased appetite and died during treatment with Fenbendazole (50 mg/kg p.o. s.i.d. for 5 days) for ascarid infection. Grossly, large areas of the oral mucosa, including the throat pouch, were ulcerated and hemorrhagic. The small intestinal mucosa was diffusely hemorrhagic and covered by loosely adherent, fibrinous membrane. Histologically, there was severe extensive fibrinonecrotic stomatitis characterized by necrosis of basal cell layer, diffuse fibrinonecrotic enterocolitis with diffuse crypt epithelial cell necrosis, and severe lymphoid depletion in the spleen. Additionally, there were a few larval Ascarids in the proventriculus and disseminated individual cell necrosis in the liver. Typical findings in avian fenbendazole toxicity include intestinal crypt cell necrosis, ulcerative stomatitis, splenic lymphoid depletion and bone marrow depression. Toxicity is associated with destruction of rapidly dividing cells via mitotic arrest at metaphase by binding of the toxin to β-tubulin and disrupting tubulin microtubule polymerization. Suspected fenbendazole toxicity has been associated with doses over 30 mg/kg p.o. s.i.d in many avian species including Columbiformes, Pscittaciformes, Ciconiiformes, and
Acciptripiformes. However, no adverse effects have been reported with doses under 25 mg/kg p.o. s.i.d in raptors, pheasants, chickens, and importantly pink-backed pelicans (*Pelecanus rufescens*) regardless of duration of administration. To the authors’ knowledge, this is the first report of suspected fenbendazole toxicity in an American white pelican.

**Poster Number:** D-42

**Section:** Diagnostic Pathology

**Keyword:** Bone and Joint

**SUSPECTED HEPATIC OSTEOODYSTROPHY IN A GOLDEN LION TAMARIN (*LEONTOPITHECUS ROSALIA*)**

E. Choi¹, S.E. Childs-Sanford², N. Abou-Madi², E.E. King⁴, B.G. Caserto¹, H. Priest³, and A.D. Miller¹

¹Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University, Ithaca, New York, USA ²Department of Clinical Sciences, Section of Zoological Medicine, Cornell University, Ithaca, New York, USA, ³Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, New York, USA, ⁴College of Veterinary Medicine, Cornell University, Ithaca, New York, USA

This report describes a case of hepatic osteodystrophy in an 8-year-old female golden lion tamarin with a six-year history of hyperbilirubinemia. Clinical examination and serum chemistries revealed hypotension, hypothermia, hypoglycemia, markedly elevated liver enzymes including serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT), and confirmed the persistent hyperbilirubinemia. In addition, complete blood count analysis was compatible with chronic lymphoid leukemia. Grossly, there was micronodular cirrhosis of the liver and diffuse osteoporosis of all examined bones. All bones as well as the kidney were diffusely pale green-yellow consistent with bilirubin deposition. Microscopic examination of the liver confirmed the micronodular cirrhosis accompanied by severe cholestasis, nodular regeneration, and foci of dysplastic hepatocytes. Histologic examination of multiple long bones revealed diffuse osteopenia, osteomalacia, and fibrous osteodystrophy. This report presents a case of chronic liver disease and lesions indicative of metabolic bone disease, also known as hepatic osteodystrophy. Hepatic osteodystrophy is not currently reported in the veterinary literature and herein we discuss potential hereditary diseases that can cause cirrhosis and mechanisms of hepatic osteodystrophy.

**Poster Number:** D-43

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Wildlife

**SYSTEMIC RANAVIRAL INFECTION IN AN AFRICAN SPURRED (*CENTROCHELYS SULCATA*) TORTOISE**

G. Krane¹, N. Steckler¹, C. Cummings², T. Waltzek¹, J. Wellehan¹, and L. Farina¹

¹University of Florida College of Veterinary Medicine, Gainesville, FL, ²Ultrapath Imaging, Durham, NC
An adult female African spurred (Centrochelys sulcata) tortoise from a large breeding facility suddenly died three weeks after three conspecifics from the same pen. Except several conspecifics from the same pen exhibiting mild upper respiratory signs, no clinical abnormalities were identified in other animals.

Numerous tan plaques covered most of the tongue and portions of oral mucosa, pharynx, and glottis. The pharynx, esophagus, and pylorus contained pinpoint, red foci.

Microscopically, there was widespread, multifocal to coalescing splenic necrosis associated with heterophilic inflammation, vasculitis, fibrinoid vascular necrosis, and fibrin thrombi. Macrophages rarely contained basophilic cytoplasmic viral inclusions. The tongue exhibited frequent mucosal/submucosal necrosis and mucosal ulceration with thick serocellular crusts. Vascular lesions and cytoplasmic inclusions were similar to those in the spleen. The kidney, lung, and submucosa/mucosa of the nasal cavity, pharynx, glottis, esophagus, stomach and small intestine displayed similar necrosis, inflammation, and vascular lesions, and the heart demonstrated vascular lesions lacking necrosis.

Quantitative PCR confirmed ranaviral etiology. MCP sequencing was 100% identical to Frog-virus 3, the type species for genus Ranavirus. In-situ hybridization confirmed endothelial and leukocytic ranaviral nucleic acids. Ultrastructural analysis demonstrated cytoplasmic 130-150 nm diameter, icosahedral viral particles.

Ranaviruses are a genus of double-stranded DNA viruses in the family Iridoviridae infecting amphibians, reptiles, and fish. In chelonians, they can cause ulcerative stomatitis, glossitis, pharyngitis, esophagitis, tracheitis, splenitis, conjunctivitis, and pneumonia. Fibrinoid vasculitis, thrombosis and rare (often undetected) basophilic, cytoplasmic inclusions distinguish ranavirus from herpesvirus. To our knowledge, this is the first report in an African spurred tortoise.

**Poster Number:** D-44

**Section:** Diagnostic Pathology Focused Group Session I

**Keyword:** Infectious Disease

**THE PATHOLOGY OF TYZZER’S DISEASE IN HORSES**

K.Fresneda, F. Carvallo, and F.Uzal
CAHFS, University of California, Davis, San Bernardino, CA

Tyzzer’s disease is produced by Clostridium piliforme, a Gram negative anaerobic organism. Little new information is available about the pathology of this disease in horses. We reviewed 47 cases of Tyzzer’s disease in horses received at CAHFS between 1991 and 2015. All of the animals were less than 4 months old; 51% of the cases were between 16 and 30 days of age. Forty six per cent were male and 53% female; 40% were Quarter horses, 32% Thoroughbred and 28% were either Hanoverian, Warm blood, Paint, Arabian, crossbred or undetermined. Forty per cent of the animals were found dead without clinical signs being observed and 60% presented one or more of the following clinical signs: lethargy, diarrhea, hyperthermia, icterus, recumbency, anorexia, seizures and respiratory distress. Gross changes included one or more of the following: hepatomegaly, pulmonary edema, acinar hepatic pattern, jaundice, splenomegaly, endocardial hemorrhage, multi-organ hemorrhages, ascites, mesenteric lymphoadenomegaly, atelectasis and hydro pericardium. Microscopically, all cases presented multifocal
necrotizing hepatitis with intracytoplasmic Gram negative filamentous rods in hepatocytes. Other microscopic lesions included colitis, typhilitis, enteritis, myocarditis, myocardial necrosis, splenic lympholysis, endocardial hemorrhages, interstitial pneumonia and DIC. Hepatic necrosis was the only consistent finding, with only a small percentage of cases showing intestinal and cardiac lesions, the other two changes considered to be part of the characteristic triad of lesions in this disease. These results confirm that the liver is the main organ to be examined in suspect cases of Tyzzer’s disease.

**Poster Number:** D-45

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**USE OF AN AZURE HISTOCHEMICAL STAIN TO ASSIST IN DISTINGUISHING BETWEEN POORLY DIFFERENTIATED ENTERIC MAST CELL TUMORS AND LARGE GRANULAR CELL LYMPHOMA**

G. Krane¹, C. Cummings², J. Abbott¹, and J.A. Conway¹
¹University of Florida College of Veterinary Medicine, Gainesville, FL, ²Ultrapath Imaging, Durham, NC

A 10 year-old castrated domestic-shorthair cat presented for acute vomiting. Abdominal exploratory was performed based on suspicion of a linear foreign body. The patient had an eosinophilic abdominal effusion. A 19 cm segment of jejunum with a stenotic lumen narrowed to 1-2 mm in diameter and walls thickened up to 4 mm was removed and submitted for histopathology.

Microscopically, sheets and cords of round cells infiltrated the submucosa and muscularis. Cells had distinct borders, little cytoplasm, and ovoid to round, multiple nuclei with variably distinct, multiple nucleoli. Anisocytosis and anisokaryosis were moderate to marked, and mitoses were zero/ten 400x fields. Cells averaged 14-21 µm and reached up to 28 µm in diameter. Low numbers of mast cells and numerous eosinophils were admixed with the mass. Numerous azurophilic (PTAH stain) cytoplasmic granules were detected in neoplastic cells. Cells infiltrated adjacent omentum and lymph node. Though mast cell tumor was initially considered as a differential, a diagnosis of large granular cell lymphoma (LGL) was made based on azurophilic granulation.

Immunohistochemistry was performed for CD3, CD79a, and c-kit. Approximately 60-70% of neoplastic cells exhibited weak (intestines) to strong (omentum), cytoplasmic CD3 immunoreactivity and no CD79a or c-kit immunoreactivity. Clonality testing for T cell antigen receptor confirmed T-lymphocyte LGL. Ultrastructural analysis is pending.

The literature regarding feline LGL is sparse. This case shows azure stains can help differentiate between enteric mast cell tumor and LGL.

**Poster Number:** D-46

**Section:** Diagnostic Pathology  
**Keyword:** Nervous System
VERMINOUS MENINGOENCEPHALITIS AND HYPOPHYSITIS: A CASE OF HALICEPHALOBUS GINGIVALIS IN A BROWN SWISS HEIFER

J. Lorbach, K. Wilson, and P. Stromberg
Department of Veterinary Biosciences, The Ohio State University, Columbus, OH

A two-year-old Brown Swiss heifer presented to the Ohio State University Veterinary Medical Center for evaluation following a two day history of neurologic signs suspicious for listeriosis. Clinical findings included lethargy, recumbency, horizontal nystagmus, absent menace response, and sluggish pupillary light reflexes. The animal failed to respond to intravenous fluids and antibiotic therapy and progressed to a moribund state. The animal was euthanized and submitted for autopsy. Gross examination was unremarkable. Histologic examination revealed extensive pyogranulomatous and eosinophilic inflammation, degeneration, and necrosis within the pituitary gland, brainstem, thalamus, cerebellum, and meninges; lesions were most severe in the pituitary gland. The microscopic lesions were centered on the vasculature, and included myriad intraslesional adult and immature larval nematodes as well as ova in various stages of development. Adult nematodes measured approximately 20 micrometers in diameter with a thin cuticle; meromyarian musculature; pseudocoelom; rhabditiform esophagus including a corpus, isthmus, and bulb; uninucleate ova; and dorsoflexed ovary. These findings are diagnostic characteristics of Halicephalobus gingivalis. Much remains unknown regarding the life cycle of this organism and pathogenesis of the disease it causes, though the distribution of lesions in the current case suggests hematogenous spread. Reports of disease associated with this organism are exceedingly rare outside of humans and equids. The findings in this case suggest H. gingivalis should be considered as a potential etiologic cause of severe progressive neurologic disease in cattle.

Poster Number: D-47

Section: Diagnostic Pathology Focused Group Session II
Keyword: Infectious Disease

EOSINOPHILIC GRANULOMATOUS DERMATITIS INDUCED BY THE OOMYCETE LAGENIDIUM SP. IN A CAT

L.K. Manning1, D.M. Hertzke2, L.V. Reiter3, and E.J. Olson1
1Department of Veterinary Population Medicine and Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, Minnesota, 2Veterinary Services, Marshfield Labs, Marshfield, Wisconsin, 3Department of Veterinary Clinical Sciences, University of Minnesota, St. Paul, Minnesota

A 3-year-old, female spayed, domestic shorthaired cat was necropsied following euthanasia for recurring dermatitis on the caudodorsum secondary to Lagenidium sp.. Tail chewing and hair loss had occurred since this cat was acquired as a kitten from Florida. Previous biopsy and culture of areas of alopecia with erythematous and crusted papules, plaques, and nodules over the dorsolateral tail and caudodorsum yielded scant mold; further identified as Lagenidium sp. by 18S/ITS nucleic acid amplification and sequence analysis. Tail amputation and wide excision of the affected skin was performed and the cat was euthanized following suspected recurrence 3 weeks following surgery. At necropsy, there were two reddened, ulcerated areas over the caudodorsum, accompanied by variable brown crusts and dried red-brown exudate. The underlying subcutis contained poorly demarcated, tan to pink, multinodular, firm
tissue. Histologically, underlying the ulcerated epidermis and expanding the dermis, there was a poorly
demarcated, non-encapsulated infiltration of large numbers of eosinophils accompanied by moderate
lymphoplasmacytic and histiocytic infiltrates, fewer neutrophils, and low numbers of multinucleated
giant cells. Multifocal areas of necrosis frequently contained small to moderate numbers of
predominately negatively stained, extracellular, 10-15 µm diameter fungal hyphae. Grocott’s
methenamine silver stain highlighted broad, thick-walled fungal hyphae that were occasionally septate,
had variably prominent bulbous dilatations, and intermittent right angle branching. There was no
evidence of spread to visceral organs. Members of the genus Lagenidium sp. are a group of
the Oomycetes (water molds), closely related to Pythium. Most published cases involve dogs; however,
several recent reports have involved cats.

**Poster Number:** D-48

**Section:** Diagnostic Pathology Focused Group Session II

**Keyword:** Infectious Disease

**RAPID DETECTION OF CWD PRIONS IN FIXED PARAFFIN EMBEDDED TISSUES USING THE REAL-TIME QUAKING INDUCED CONVERSION ASSAY**

C.E. Hoover, D.M. Henderson, M. Zabel, and E.A. Hoover
Prion Research Center, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

Traditional diagnostic detection of chronic wasting disease (CWD) in post-mortem cervid tissues relies
on identification of misfolded CWD prion protein (PrP\text{CWD}) by western blotting, ELISA, or
immunohistochemistry (IHC). These techniques require two separate sample collections (frozen and
fixed) which may lead to discrepancies in results due to variation in CWD prion tissue distribution and
assay sensitivities. In addition, these methods require that a substantial amount PrP\text{CWD} be present in
tissues, which can limit CWD detection in early and subclinical infections. A recently introduced prion
detection assay, the real-time quaking induced conversion assay (RT-QuIC), uses prion-specific amyloid
conversion to enhance detection in samples with low prion burdens. Here we describe a method to
analyze paraformaldehyde-fixed paraffin embedded tissue sections for the presence of CWD prions. We
found the fixed tissue real-time conversion assay (fRT-QuIC) has greater sensitivity of CWD detection
than IHC alone, the current gold-standard detection method, thus providing enhanced CWD
identification in a variety of tissues with a low prion burden including those that are IHC-negative. In
ongoing work, we also found that fRT-QuIC amyloid amplification kinetics can be used to provide a semi-
quantitative estimate of prion concentration in a given tissue sample. By combining IHC analysis of
PrP\text{CWD} distribution and fRT-QuIC quantification data, we are now able to describe both CWD location
and estimate CWD infectivity in a given tissue sample. This technique has the potential to greatly
enhance diagnostic and investigative detection of CWD prions in subclinical infections and historical
paraffin-embedded samples.

**Poster Number:** D-49

**Section:** Diagnostic Pathology

**Keyword:** Alimentary
A CASE OF FELINE GASTROINTESTINAL EOSINOPHILIC SCLEROSING FIBROPLASIA IN SINGAPORE: HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION

C.B. Ong, M.K. Kosnan, F.M. Ibrahim, and M. Al-Haddawi
Advanced Molecular Pathology Laboratory, Institute of Molecular and Cell Biology, A*STAR, Republic of Singapore

Feline gastrointestinal eosinophilic sclerosing fibroplasia (FGESF) is a new disease entity first reported in 2009 affecting the gastric pyloric, ileocecal junction or colonic regions in cats of a wide age range (14 weeks to 17 years). It has been reported with poor prognosis in cats of longhaired breed, in USA, Europe, Japan and New Zealand, with no conclusive etiologic causative agent(s). This report describes the first case of FGESF in Singapore in a 9-months-old intact Persian female cat with a history of intermittent vomiting and failure to gain weight. Grossly, pylorus is pale with roughen mucosal surface. Microscopically, there are intense fibroproliferative anastomosing cords and trabeculae of collagen separated by reactive fibroblasts, eosinophils and mixed inflammatory cells. Special stains exhibited bundles of collagen fibers (masson trichrome), scattered to regionally dense mast cells (Toluidine blue) among the fibroplasia, clusters of intralesional gram positive and negative bacteria (Brown&Brenn) and absence of fungal agents (PAS). Immunohistochemically, the fibroblastic population is strongly and diffusely positive for vimentin and smooth muscle actin, and small caliber blood vessels are strongly positive for CD31. Moderate numbers of lymphocytes are positive for CD3 for which are concentrated towards the luminal aspect of the transmural lesion. The cat died 1.5 months post-diagnosis despite supportive treatment. Current findings indicate that FGESF can occur in a fairly young cat, and is a fibroproliferative lesion characterized by extensive angiogenesis, smooth muscle differentiation, associated with mixed inflammatory cells of T-lymphocytes, mast cells and eosinophils. Further immunohistochemical staining is recommended to investigate this disease.

Poster Number: D-50

Section: Diagnostic Pathology
Keyword: Neoplasia

A CASE REPORT OF YOLK SAC CARCINOMA IN PULMONARY ARTERY IN A YOUNG FEMALE SPRAGUE-DAWLEY RAT

Y. Sakamoto1, T. Nagaoka2, K. Tamura1, and H. Kaneko1
1Teijin Pharma Ltd. Hino-shi, Tokyo, Japan, 2Shin Nippon Biochemical Laboratories, Kagoshima-shi, Kagoshima, Japan

Yolk sac carcinoma is a rare tumor in rats usually noted in the genital system, particularly in ovaries of older animals. We observed a yolk sac carcinoma in the pulmonary artery in a treated 18-week-old female SD rat from a 4-week repeated intraperitoneal dose toxicity study. The rat was found dead at day 76 on test (during the recovery period at the 55th day after the final dosing). At the necropsy small white nodules were present on the lung surface. Tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sections were stained with hematoxylin-eosin and periodic acid Schiff (PAS).
Tumor emboli were observed in the vasculature of the pulmonary artery. Tumor cells were characterized by slightly basophilic vacuolated cytoplasm and large vesicular nuclei with prominent nucleoli. They formed nests or clusters which were embedded in a homogenous eosinophilic, PAS positive matrix. The tumor cells and matrix were positive for laminin immunohistochemistry. These features were compatible with reports of parietal pattern of yolk sac carcinoma in rodents. Following a detailed review, no primary tumor masses were identified in the reproductive system or in any other organs. This tumor was considered to be spontaneous since it was only 1 case in the study and the presence of the tumor in such a young animal seemed too short to develop from chemical administration.

**Poster Number:** D-51

**Section:** Diagnostic Pathology  
**Keyword:** Forensic Veterinary Pathology

**A DOUBLE HOMICIDE: MAN AND HIS BEST FRIEND**

A. Stern  
Veterinary Diagnostic Laboratory, University of Illinois, Urbana, IL

A man and a dog were found dead in a house upon extinguishing a house fire and foul play was suspected based on findings at the scene. An adult, female, German Shepherd dog was submitted to the University of Illinois Veterinary Diagnostic Laboratory for postmortem (forensic) examination. Within the left side of the neck there were multiple stab wounds and incised wounds extending into the skeletal muscle of the neck. Stab wounds each had a single blunt margin and a single sharp margin. The left carotid artery and jugular vein was intact. Within the oral cavity, larynx, trachea, and esophagus there was black material consistent with soot. Carboxyhemoglobin levels were performed and was 77% with postmortem blood. Hydrogen cyanide was negative with postmortem blood. Based on postmortem examination the cause of death is due to a combination of sharp force injury and inhalation of toxic fire fumes. Autopsy findings of the human being were consistent with sharp force injuries and the manner of death was determined to be homicide.

**Poster Number:** D-52

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**A HIGH-GRADE MYXOID LIPOSARCOMA IN A DOG**

Q.D. Plumlee$^{1,}$, A. Hernandez$^{1,}$, S. Clark$^{1,}$, A. Bascunan$^{2,}$, J. Davidson$^{2,}$, and J. Mansell$^{2}$  
$^{1}$ Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, $^{2}$ Department of Veterinary Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

This case report describes a 10-year-old, neutered male, basset hound with a 26x21x21 cm, infiltrative mass on the left abdominal wall that did not extend into the peritoneal cavity based on radiographs and
abdominal CT. Cytologic examination revealed moderate numbers of neoplastic round cells, which frequently contained numerous round, clear, cytoplasmic vacuoles. Histologically, the neoplasm was composed of two morphologically distinct cell populations forming a continuum of heterogeneously differentiated cells. The primary population formed streams and sheets, with abundant, lightly eosinophilic alcian blue positive myxoid matrix. The cells were spindloid and multifocally contained intracytoplasmic, clear vacuoles. The second population was arranged in sheets and had a round cell appearance with variably vacuolated cytoplasm. Vacuoles within both populations were osmium positive (lipid). Multifocal large pools of mucin formed pseudocysts, and numerous small capillaries were throughout the neoplasm. As defined by the current WHO veterinary classification of liposarcomas, this neoplasm has morphologic features of both the myxoid and pleomorphic liposarcomas, however it is analogous to the recently defined high-grade myxoid liposarcoma in humans. The 2013 WHO classification of liposarcomas in humans defines a high-grade myxoid liposarcoma as containing prominent myxoid stroma with the presence of solid sheets of round neoplastic cells within the neoplasm. To our knowledge a myxoid liposarcoma with round cells has not been previously described in dogs, and only one report has been described in a macaque. Furthermore, this case highlights the need to potentially re-evaluate the current classification of liposarcomas in veterinary species.

**Poster Number:** D-53

**Section:** Diagnostic Pathology  
**Keyword:** Forensic Veterinary Pathology

**A REVIEW OF THE CAUSE AND MANNER OF DEATH OF 100 FORENSIC NECROPSY CASES**

A. Stern and E. Asrow

Forensic veterinary pathology is increasingly becoming a major component of a legal investigations involving animal abuse and neglect. A search of the computer records for the 100 most recent medicolegal cases (forensic necropsies) submitted to the Veterinary Diagnostic Laboratory, University of Illinois was performed. Based on a review of the pathology records, submission information, and available court records, cases were categorized by signalment, cause of death, and manner of death (MOD) or manner of injury (MOI) if the animal was euthanized. The cause of death was determined in 90% of forensic necropsy cases. The most commonly submitted animal species were dogs (63%) and cats (17%). The most common causes of death were complications of negative caloric intake (16%), blunt force trauma (9%), sharp force injury (9%), and projectile injuries (5%). Case findings were consistent with a non-accidental MOD/MOI in 51% of cases, accidental MOD/MOI in 19% of cases, natural disease MOD/MOI in 15% of cases, therapeutic complication MOD/MOI in 4% of cases, and undetermined MOD/MOI in 9% of cases.

**Poster Number:** D-54

**Section:** Diagnostic Pathology  
**Keyword:** Urinary System
BENIGN AND MALIGNANT RENAL VASCULAR TUMORS IN CAPTIVE AGED CHIMPANZEES (*PAN TROGLODYTES*) FROM ALAMOGORDO PRIMATE FACILITY

J. Chilton\(^1\) and M. Lammey\(^2\)
\(^1\)Charles River, Reno, Nevada, \(^2\)Alamogordo Primate Facility, Alamogordo, New Mexico

As the population of geriatric chimpanzees in captivity increases, their age-related diseases are becoming apparent, including neoplasia. Data of prevalence of kidney tumors in chimpanzees are valuable for continued management of the colonies. Tumors of vascular origin are rare in chimpanzees. Previously, benign hemangiomas have been reported in skin (n=1), spleen (n=1), and kidney (n=2) of chimpanzees, but no malignant renal vascular tumors have been described. Three cases of spontaneous renal vascular tumors occurred in the aged chimpanzees at Alamogordo Primate Facility between 2001 and 2015. The vascular origin of the tumors was confirmed by positive anti-CD31 immunohistochemistry. The tumors were diagnosed as hemangioma (n=1, female, age 53) and hemangiosarcoma (n=2, males, age 21 and unknown). In both cases of hemangiosarcoma, there were foci of tumor in the adrenal glands. Whether this represents multicentric disease similar to renal angiosarcomadescribed in humans or metastasis of the primary kidney mass is uncertain.

**Poster Number:** D-55

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

BOVINE “WINTER DYSENTERY” OUTBREAK DURING THE SUMMER OF SOUTHERN CALIFORNIA

S. Diab  
California Animal Health and Food Safety Laboratory, University of California, Davis, CA

Bovine coronavirus (BoCV), a *Betacoronavirus* 1, has been associated with acute, often hemorrhagic, diarrhea in beef and dairy cattle almost exclusively during the cold months of the year; therefore the disease is commonly known as “winter dysentery”. We report a large outbreak of “winter dysentery” in dairy cattle that occurred during early August in Southern California (San Diego County) with an average daily high temperature of 27° Celsius and average low temperature of 20° Celsius during this month. Fifty out of 500 Holstein cows presented with lethargy, decreased appetite and hemorrhagic diarrhea during a 2-week period. Four cows died and two were submitted for necropsy. Grossly, both cows had distended spiral and descending colon and rectum, which were filled with abundant hemorrhagic, finely chopped, moist digesta admixed with scant amount of clotted blood. The mucosa of the colon and rectum was diffusely dark red. Histologically, there was severe, hemorrhagic, necrotizing colitis and proctitis with crypt abscesses, necrosis of superficial epithelium, and crypt blunting and fusion. Colon and rectum samples from both cows were positive for BoCV by fluorescent antibody test, immunohistochemistry, rtPCR and Coronavirus-like viral particles were detected by direct electron microscopy in fecal samples. Tests for *Salmonella* (rtPCR), bovine viral diarrhea virus (rtPCR and immunohistochemistry), *Yersinia* (cold enrichment culture), Rotavirus (antigen ELISA) and anticoagulant
screen were all negative and no parasite eggs were observed on fecal floats in both cows. “Winter dysentery” should be considered a differential diagnosis for hemorrhagic diarrhea in cattle even during the summer time.

**Poster Number:** D-56

**Section:** Diagnostic Pathology  
**Keyword:** Muscle

**BOVINE FETAL EMPHYSEMA ASSOCIATED WITH IN UTERO CLOSTRIDIUM CHAUVOEI INFECTION**

E.E. Edwards¹, J.F. Edwards¹, F.A. Uzal², and R.R. Rech¹  
¹College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX,  
²California Animal Health and Food Safety Laboratory System, University of California, Davis, CA

A 2-year-old, pregnant, Gyr cow from an unvaccinated herd presented to the Texas A&M University Veterinary Teaching Hospital for recumbency. On physical examination subcutaneous emphysema was palpated in the hind limbs, and cytologic aspirates revealed bacterial rods consistent with *Clostridium chauvoeï* morphology. The cow was humanely euthanized with a diagnosis of blackleg. On postmortem examination, extensive necrohemorrhagic myositis, worse in the hindlimbs, and a fibrinous epicarditis with myocardial necrosis were noted. A gas-distended and taut uterus contained a near-term, markedly emphysematous fetus. The fetus contained pleural fibrin clots, and a fibrinohemorrhagic pneumonia was histologically demonstrated. Numerous gram-positive bacilli were found in multiple tissues from both the dam and the fetus. Anaerobic culture and fluorescent antibody tests performed on skeletal muscle from both animals were positive for *Clostridium chauvoeï*. The fetus was presumably infected *in utero* through hematologic spread of clostridium spores. Blackleg is classically a disease of young, fast growing cattle and has not been described in a bovine fetus. Fetal emphysema has historically been reported in sheep with clostridiosis; however, to our knowledge has never been reported in cattle. This represents a unique case of *in utero* blackleg infection.

**Poster Number:** D-57

**Section:** Diagnostic Pathology  
**Keyword:** Reproductive System

**BOVINE NEOSPOROSIS AND ABORTIONS IN BRITISH COLUMBIA, CANADA**

S.Raverty, D. Wilson, K. Orsel, J. Waddington, M. Rajeev, A. Sweeney, T. Joseph, and M. Grigg

Reproductive loss is a significant management concern with dairy cattle. To assess the importance of bovine neosporosis as a cause of abortions in British Columbia, a retrospective study of pathology records of bovine fetal submissions to the Animal Health Center, Abbotsford, BC was undertaken. Between January 2007 and July 2013, there were 182 bovine fetal accessions and from July 2013 to May 2014 active case recruitment was initiated from local producers resulting in 54 abortion
submissions. Fetuses presented for necropsy underwent a standardized post mortem examination and cases were confirmed by histopathology with one or more positive test results by immunohistochemistry, serology (cELISA, Pullman, WA), or polymerase chain reaction for Apicomplexa (Np21/Np6 and ApiITS1 primers). Myocarditis, myositis and encephalitis were the most common microscopic findings and neosporosis was confirmed in 19.1% of submitted fetuses from 2007-2014. Between January 2007 and July 2013, 14.4% of the abortions were diagnosed as neosporosis. In contrast, with active case recruitment of fetal abortions between July 2013 and May 2014, the incidence of neosporosis increased to 40.9% of fetal loss. Neosporosis was most commonly identified in fetuses between 3 to 6 months gestation and there was no significance associated with dam parity. Some differences in diagnoses may be attributed to test sensitivity and specificity. Active surveillance established that immediate submission of bovine abortion samples dramatically improved diagnosis and detection of Neospora caninum as a significant causal agent of bovine abortion and production losses in the upper Fraser Valley, a region where neosporosis is highly prevalent.

**Poster Number:** D-58

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

**CANINE HERPESVIRUS-1-INDUCED ACUTE SYSTEMIC RESPIRATORY DISEASE IN THREE CLINICALLY HEALTHY ADULT DOGS WITH EVIDENCE OF HORIZONTAL TRANSMISSION**

S. Yang and J. Cooley  
Mississippi State University, College of Veterinary Medicine, Starkville, MS

Canine Herpesvirus-1 is known to cause fatal systemic infections in neonatal puppies, usually less than 2 weeks of age. Clinical signs in immunocompetent adult dogs are typically mild, self-limiting and localized to genitalia, eyes and upper respiratory tract. This case report documents 3 healthy adult dogs (11-year-old Dalmatian, 9-year-old English bulldog, 7-year-old French bulldog) infected with Canine Herpesvirus-1 resulting in acute systemic disease with respiratory tract and ocular manifestations. The initial case was an 11-year-old Dalmatian euthanized due to severe respiratory distress. Pathologic examination revealed hemorrhagic bronchointerstitial pneumonia and multiorgan necrosis. This dog was positive for CHV-1 by PCR and virus isolation, culture negative and negative for other canine viral respiratory pathogens. The Dalmatian was identified as the source of nosocomial infection of the other dogs. The 9-year-old English bulldog was transiently in contact with the Dalmatian and developed similar respiratory and ocular signs 10 days later. The housemate of the English bulldog was infected 8 days subsequent to this. Both dogs were positive for CHV-1 by PCR and viral isolation, treated with antivirals and survived. The English bulldog was on maintenance cyclosporine for atopy; the French bulldog had no predisposing factors. Only 5 other cases of CHV-1-induced acute systemic respiratory infection in healthy adult dogs have been previously reported. Four of these are recent cases in Georgia and Mississippi. Recrudescence of existing viral infection is not suspected in these cases. Thus, CHV-1 can result in acute fatal infection in clinically healthy adult dogs and is contagious by contact.

**Poster Number:** D-59
**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**CCDC85C EXPRESSION IN MAMMARY GLAND TUMORS IN ANIMALS**

N. Tanaka, T. Izawa, J. Yamate, and M. Kuwamura  
Osaka Prefecture University, Izumisano, Osaka, Japan

A mutation in coiled-coil domain containing 85c (Ccdc85c) gene causes non-obstructive hydrocephalus and subcortical heterotopia in mice. Ccdc85C protein localizes at cell-cell junction of various proliferating simple epithelia including neuroepithelial cells. Thus we hypothesize that Ccdc85C plays a critical role for proliferative capacity in the simple epithelia. We examined expression pattern of Ccdc85C in mammary gland tumors of several animal species.

Immunohistochemistry for Ccde85C revealed Ccde85C expression was observed in the mammary gland tumors of dogs, cats and rats. In the neoplastic luminal epithelial cells, Ccde85C was strongly expressed at apical junctions as well as simple epithelia of normal organs. However, canine carcinoma cells without obvious acinar structure tended to have ubiquitous expression at cell-cell junctions.

These findings suggested Ccde85C expression may be related to histogenesis and malignancy of mammary gland tumors.

**Poster Number:** D-60

**Section:** Diagnostic Pathology  
**Keyword:** Nervous System

**CEREBELLOMEDULLARY NEURONAL DEGENERATION IN A HAVANESE PUPPY: A NEW SYNDROME?**

J. Perkins¹, J.H. Hammond¹, B.A. Summers², and A.D. Miller³  
¹Pieper Memorial Veterinary Center, Middletown, CT, ²University of Melbourne, Melbourne, Australia, ³Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, Section of Anatomic Pathology, Ithaca, NY

A 5-week-old female intact Havanese puppy presented with a gait deficit since birth and pelvic limb weakness compared to the thoracic limbs. When supported, the puppy had a cerebellar ataxia. Reflexes and tone were decreased in the pelvic limbs. The neuroanatomic diagnosis was cerebellum with suspect additional involvement of the neuromuscular system. Gross examination of the brain revealed a small cerebellum. Histologic evaluation of the central nervous system (CNS) revealed widespread Purkinje cell loss while those remaining were degenerate characterized by hypereosinophilia, cell swelling and vacuolation, cytoplasmic mineralization, and loss of cell processes. Moderate astrogliosis was present in the depleted Purkinje cell layer and the molecular layer was narrowed with mild gliosis. Further, neuronal chromatolysis and secondary gliosis were noted in the medial and caudal vestibular nuclei as well as the cerebellar fastigial, interposital, and lateral nuclei. Other brainstem nuclei were spared. Small numbers of chromatolytic neurons were present in
the spinal cord (only cervical region available), most noticeable in the ventral horn. No histologic lesions were noted in non-CNS tissues. This case is unusual due to the severity of the Purkinje cell degeneration, loss and dense gliosis occurring at such a young age coupled with degenerative changes in cerebellar and vestibular nuclei. Systems degeneration syndromes affecting multiple, functionally related nuclei and tracts within the CNS, are uncommonly reported in veterinary medicine. These diseases typically manifest with early onset cerebellar degeneration coupled with degenerative changes in multiple nuclei within the CNS.

Poster Number: D-61

Section: Diagnostic Pathology
Keyword: Nervous System

CHARACTERIZATION OF NEUROPATHOLOGICAL CHANGES IN MURINE MODELS OF MUCOPOLYSACCHARIDE STORAGE DISEASE

N. Robinson

Department of Population Medicine, College of Veterinary Medicine, University of Minnesota Mucopolysaccharide storage (MPS) disorders are a group of lysosomal storage disorders characterized by the accumulation of glycosaminoglycans within the lysosome resulting in cellular and organ dysfunction. A common characteristic of several mucopolysaccharide storage disorders is neuropathological lesions. In the current study, clinically normal as well as congenic C57BI/6 mice carrying a targeted mutation in either the idua (MPS I), Naglu (MPS IIIB), or GusB (MPS VII) gene underwent histological analysis of the brain. Coronal sections through the cerebrum, hippocampus, and cerebellum of brains fixed in 4% paraformaldehyde were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) or periodic acid-schiff (PAS) + alcian blue and evaluated using light microscopy. Purkinje cells in all three MPS mouse strains were swollen and contained numerous PAS positive intracytoplasmic vacuoles. Additionally, astrocytes, cortical neurons and perivascular cells contained similar PAS positive vacuoles in all three sections evaluated. However, the size and number of vacuoles in affected cells of mice with the Naglu gene deficit (MPS IIIB) were subjectively reduced compared with the other two genes. Additionally, the hippocampal neurons appears largely unaffected in the Naglu (MPS IIIB) and GusB (MPS VII) mice. Our study provides characterization of neuropathological changes in murine models that are currently being used to study MPS disorders. These results contribute an important foundation for evaluating and monitoring the efficacy of novel viral vector based gene therapies and supports the relevance of murine models for mucopolysaccharidosis research.

Poster Number: D-62

Section: Diagnostic Pathology
Keyword: Infectious Disease
CLOSTRIDIUM DIFFICILE COLONIC INFECTION IN A WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) WITH CAPRINE HERPESVIRUS-2-ASSOCIATED MALIGNANT CATARRHAL FEVER

F. Giannitti, H. Li, D. Patnayak, K. Olsen, B. Crossly, H. Moore, and F. Uzal

Two white-tailed deer developed ataxia and died two weeks after being introduced into a herd in Texas. A necropsy performed in one case, a 1-year-old female, revealed fluid colonic contents, suggesting diarrhea. Tissues (colon, intestine, kidney, liver, heart, spleen) and colonic contents were submitted to the UMN-VDL.

Histologic examination revealed multifocal necrotizing enterocolitis with mucosal microthrombosis and scattered crypt microabscesses. Clostridium difficile was isolated and A/B toxins were detected in colonic contents (ELISA). Additionally there was multifocal moderate lymphohistiocytic interstitial nephritis with necrotizing arteries (arcuate arteries), and mild lymphocytic portal hepatitis. The necrotizing arteritis prompted malignant catarrhal fever (MCF) investigation. A frozen sample (pooled kidney, liver, heart, spleen) submitted to WSU was positive for Caprine Herpesvirus-2 (CpHV-2) and negative for Ovine Herpesvirus-2, White-tailed deer-MCF virus, Alcelaphine Herpesvirus-1 and 2, and Ibex-MCF virus by multiplex PCR.

Bovine coronavirus, Rotavirus A-B, and BVDV PCRs, and Salmonella and Yersinia cultures were negative in the intestine/colon. No parasitic ova/oocysts were observed in colon contents (flotation).

The diarrhea and enterocolitis in this deer were suggestive of C. difficile-associated disease, which to our knowledge has not yet been described in cervids. Renal lesions were consistent with MCF, and CpHV-2-induced MCF was confirmed. Whether C. difficile was a primary or secondary pathogen is unknown. We speculate that C. difficile could have proliferated and produced A/B toxins in a debilitated individual suffering from MCF. However, a primary pathogenic role cannot be excluded. The role of C. difficile as a cause of enterocolitis in deer should be further explored.

Poster Number: D-63

Section: Diagnostic Pathology
Keyword: Neoplasia

COMPARISON OF CUTANEOUS MAST CELL TUMOUR GRADING IN THE HISTOLOGICAL TWO-TIER GRADING SYSTEM WITH Ki-67 GRADING

M. Van Erp, R. de Kroon, C. Dirken, and J.S. McKay
Idexx Laboratories Ltd., United Kingdom

Prognostic determination for canine cutaneous mast cell tumours (MCTs) utilises two grading systems, one recommended by Patnaik et al. 1984 and the two-tier grading system (Kiupel et al. 2011). Ki-67 immunohistochemistry is also used to give further prognostic indication in grade II tumours. The purpose of this study was to review MCTs chosen at random over three months within our database,
and to investigate whether the Ki-67 scoring system of immunohistochemically low grade or immunohistochemically high grade (Scape et al., 2006) correlated with a low grade or high grade Grade II mast cell tumour in the two-tier system. The review included 182 cutaneous mast cell tumours in dogs (Female: 99 and Male: 83). Out of a total of 182 mast cell tumours, 166 were graded by microscopy with the two-tier system as low grade, while 16 were high grade. Ki-67 results revealed a 71% concordance for the diagnosis of Grade II, low grade (two-tier system) and immunohistochemically low grade (Ki-67 grade), and a concordance of 81.25% for Grade II, high grade, and immunohistochemically high grade (Ki-67 grade). These results indicate that high grade, grade II mast cell tumours have a higher correlation with their respective Ki-67 grading, thus indicating that a high grade tumour benefits less from additional Ki-67 testing in providing a prognosis to clients. In contrast, the benefit of performing Ki-67 on low grade tumours is greater and may highlight additional cases which have a tendency towards a poorer prognosis.

Poster Number: D-64

Section: Diagnostic Pathology
Keyword: Infectious Disease

DEMONSTRATION OF SPONTANEOUS TRANS-PLACENTAL TRANSMISSION OF OVINE HERPESVIRUS TYPE 2 IN CATTLE WITH SHEEP-ASSOCIATED MALIGNANT CATARRHAL FEVER

S.A. Headley¹, L.A. Pimentel¹, V.H.S. Oliveira², H.S. Toma¹, A.F. Alfieri², A.M. Carvalho¹, M.D. dos Santos¹, and A.A. Alfieri²
¹Universidade de Cuiabá, Mato Grosso, Brazil, ²Universidade Estadual de Londrina, Paraná, Brazil

Sheep-associated malignant catarrhal fever (SA-MCF) is an important infectious disease of ruminants worldwide that is caused by ovine herpesvirus 2 (OvHV-2). OvHV-2 is transmitted predominantly by contact between infected and susceptible hosts, while the documentation of vertical transmission is rare. This study documented the pathological and molecular findings associated with transplacental transmission of OvHV-2 in cattle. Two Girolanda cows with corneal edema, apathy, mucopurulent discharge, and ulcerative stomatitis died spontaneously; one of these was pregnant to a 4-month-old fetus. Significant pathological findings included widespread lymphoplasmacytic necrotizing vasculitis and lymphoplasmacytic accumulations in the kidneys, carotid rete miriabile, brain, lymph nodes, and lungs of both cows. Lesions in the fetus included lymphoplasmacytic necrotizing vasculitis and myocarditis and accumulations of lymphoplasmacytic inflammatory cells within the kidneys and portal regions of the liver. A PCR assay that targets the tegument protein gene of OvHV-2 amplified viral DNA from the brain of the pregnant cow and her fetus, as well as from the kidney of the pregnant cow. PCR assays designed to identify bovine herpesvirus type 1 (BoHV-1) and BoHV-5 were negative. In conclusion, pathological alterations consistent with SA-MCF were identified within multiple tissues of two cows and a four-month-old fetus of one of these. Additionally, the amplification and sequencing of OvHV-2 from brain tissues of the cow and its fetus confirmed the participation of this pathogen in the lesions observed in these animals and demonstrated the transplacental transmission of OvHV-2 in SA-MCF of cattle.

Poster Number: D-65
ESTIMATING SHOOTING DISTANCE IN GUNSHOT WOUNDS TO ANIMALS

J.W. Brooks¹, R. Ristenbatt², I. Gronchi², S. O’Brien², M. Lyman², and K. Mantz²
¹Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA, USA, ²Forensic Science Program, Eberly College of Science, The Pennsylvania State University, University Park, PA, USA

Gunshot wounds are a common injury in domestic animals and wildlife submitted for necropsy. It is often important to the investigation to estimate the distance from which the shot was fired. Although shooting distance has been classically defined for human victims by patterns of gunshot residue on glabrous skin, little has been reported on the interpretation of findings in densely haired skin, such as animal hide. Sections of cattle hide and rabbit hide collected at slaughter were shot with four different handguns at increasing distances and evaluated for evidence of gunshot residue by multiple methods. Firearms used were .38 caliber revolver, and semi-automatic pistols in 9 mm, .40 caliber, and .45 caliber. Distances fired were contact, 1 inch, 2 inches, 4 inches, 6 inches, 8 inches, 12 inches, 24 inches, and 36 inches. Hides were initially evaluated by visible examination using stereomicroscopy under various wavelengths of light using an alternate light source. Following visual examination, hides were evaluated by radiography. Finally, hides were analyzed chemically for gunshot residue using the modified Griess test and the sodium rhodizonate test. These analyses were evaluated for their use in estimating the approximate range of fire of a gunshot wound in haired skin and were found to be useful in estimating shooting distance.

EXTRA-GASTROINTESTINAL STROMAL TUMOR FROM THE MESENTRY OF SMALL BOWL IN A DOG

T. Furukawa¹, A. Shiotuki¹, K. Nibe², K. Ono², and H. Hirao²
¹Japan Animal Refferal Medical Center, Nagoya, Aichi, Japan, ²Japan Animal Refferal Medical Center, Kawasaki, Kanagawa, Japan

A 6-year-old, neutered female Miniature Dachshund presented with fever, elevation of CRP, and a large abdominal mass. On abdominal ultrasonic examination, a well-defined large mass located in the caudal space of the right kidney was detected. Although fine needle cytology was not diagnostic, tentative diagnosis of suture-reactive granuloma was made. During the exploratory laparotomy, a large capsulated mass (7.5 x 6.3 x 3.7 cm) was observed in the duodenal mesentery, with adhesion to the omentum, pancreas and superior mesenteric artery. There was no communication with the gastrointestinal tract. Histologically, the mass was composed of spindle cells arranged in interlacing bundles with frequent formation of cystic spaces. Anisocytosis and anisokaryosis were prominent with low mitoses and no evidence of vascular invasion. The neoplastic cells showed immunolabelling for KIT
and S-100, and were negative for myogenic markers (desmin and actin) and neurofilament. On morphological and immunohistochemical findings, the mass was consistent with extra-gastrointesinal stromal tumor (EGIST) from the mesentry of the small bowl. KIT gene mutation was not detected on Exon 8, 9, and 10. The EGISTs in human are originated from the mesentry, omentum, or peritoneum, and completely separated from gastrointestinal tract wall, however their histological and immunohistochemical characteristics are identical to those of GIST. In this case, the large well-defined abdominal mass showed no communication with the gastrointestinal tract wall and similar histologic and immunohistochemical findings to those of GIST. To the authors’ best knowledge, this is the first case of EGIST in a dog.

**Poster Number:** D-67

**Section:** Diagnostic Pathology  
**Keyword:** Forensic Veterinary Pathology

**FELINE DECAPITATION: PRE-MORTEM CAUSE OF DEATH OR POST-MORTEM MUTILATION?**

A. Keggan and S. McDonough  
Cornell University, Ithaca, NY

Animal decapitation is a form of both animal cruelty and interpersonal violence, often with the purpose to intimidate, seek revenge, or instill fear in another person. Decapitation may also take place during satanic rituals. The rationale for examining cases of animal decapitation was to identify patterns associated with pre-mortem versus post-mortem decapitation. We reviewed two cases of feline decapitation that presented initially to the ASPCA then to the Anatomic Pathology Service at Cornell University. Case 1 was a decapitated kitten found by a young female child. At necropsy, severe tissue pallor was noted, indicating likely exsanguination. Lack of hemorrhage at the cut site was also noted, making it difficult to say with certainty this cat was decapitated pre-mortem. Case 2 was of a cat decapitated during a violent rampage by a family member. Hemorrhage was identified histologically in the cut tissues, along with a small amount of acute hemorrhage in the trachea. In both cases, it was challenging to say by necropsy alone that the cats were decapitated pre-mortem. Thus, pallor of mucous membranes and body tissues, hemorrhage at the cut site, and aspirated blood or hemorrhage within the tracheal lumen help to provide evidence of pre-mortem decapitation. However, the lack of one or more of these does not rule out pre-mortem decapitation. Ultimately, in deciding the time of death in cases of decapitation, the knowledge gained by necropsy must be interpreted with knowledge of the scenario under which the decapitation occurred.

**Poster Number:** D-68

**Section:** Diagnostic Pathology  
**Keyword:** Nervous System

**FLOURU-JADE C: A MARKER FOR NEURONAL DEGENERATION IN EQUINE NEONATAL MALADJUSTMENT SYNDROME**
R.E. Ruby, A.D. Miller, and T. Southard
Cornell University College of Veterinary Medicine, Department of Biomedical Sciences, Section of Anatomic Pathology, Ithaca, NY

Equine neonatal maladjustment (NMS) is estimated to affect up to 5% of equine neonates and is associated with a variety of clinical manifestations, including seizures, obtundation, and abnormal vocalization. The pathogenesis of NMS is presumed secondary to hypoxemia in utero or during parturition. Treatment requires significant financial commitment as well as intensive nursing care; therefore, NMS is a common cause of death or reason for euthanasia in newborn foals. Postmortem examination of foals with clinical manifestations of NMS is often unrewarding. The aim of this study was to investigate the utility of Flouro-Jade C (FJC) as a marker of degenerate neurons and neuronal injury in foals with clinical NMS. A retrospective search of Cornell Anatomic Pathology records for neonatal foals submitted for necropsy with clinical signs of NMS was performed and yielded 13 foals ranging from 1 to 11 days of age submitted from 1991-2014. Sections of brain were examined for histologic evidence of encephalopathy and sections of brain at the level of the hippocampus were stained with FJC. A foal with overt neuronal necrosis and a foal euthanized for spinal trauma were used as positive and negative controls to validate FJC. 10/13 foals had no histologic abnormalities with standard hematoxylin and eosin. 7/13 foals had positive fluorescence of neurons with FJC. These results suggest FJC is a useful as a marker of neuronal degeneration in horses with CNS disease that lack histologic evidence of encephalopathy and shows utility in investigating the pattern of neuronal changes in foal with NMS.

**Poster Number:** D-69

**Section:** Diagnostic Pathology

**Keyword:** Avian

**HAEMOPROTEUS MELEAGRIDIS IN AN OCELLATED TURKEY (MELEAGRIS OCELLATA)**

H. Grodi¹, M. Davis², and L. Farina¹
¹Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, ²Arcadia Animal Hospital, Arcadia, FL

A fully vaccinated, 3 year old, male ocellated turkey (*Meleagris ocellata*) was found dead inside his enclosure 19 days after being transported to Florida from a zoo located in another state. The turkey was introduced to 2 adult hens 10 days earlier, and had also appeared slightly off with decreased feed consumption on the previous day. On necropsy, the turkey was well-conditioned with adequate adipose stores. All skeletal muscle was mildly to moderately friable and had multifocal pinpoint-wide white streaks and small foci of hemorrhage. On gross examination, the lesions identified within the skeletal muscle of the turkey were thought to be sarcocysts; however, histological evaluation determined that they were most compatible with megaloschizonts of *Haemoproteus meleagridis*. Throughout the skeletal muscle were moderate to large numbers of approximately 50-300 µm megaloschizonts with areas of degeneration, necrosis and minimal inflammation. Megaloschizonts were also present in sections of myocardium with no associated necrosis or inflammation. The parasite may be transmitted to susceptible turkeys through the saliva of biting flies, such as *Culicoides sp.*., after they have taken blood meal from an infected bird. *H. meleagridis* is a hemoplasmodian found within blood cells and tissues of wild and domestic turkeys. Hemoplasmodia are microscopic, intracellular parasitic protozoans
that are closely related to the genera of Plasmodium and Leucocytozoon. In some cases, infections in highly susceptible individuals can result in death.

**Poster Number:** D-70

**Section:** Diagnostic Pathology  
**Keyword:** Toxicologic Pathology

**IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LYMPHOCYTIC AND GRANULOMATOUS LESIONS IN BEEF CATTLE WITH CITRUS PULP TOXICITY**

W.L. Castleman and M.T. Long

Sudden onset of disease with death was reported in 17 of 200 Brangus cattle on Florida scrub pasture that were supplemented with grain concentrate and allowed free choice to citrus pulp as the main source of roughage. Clinical signs noted in cattle included thin body condition, alopecia and poor hair coat, anemia and ataxia. Laboratory tests confirmed anemia and elevation of liver enzymes. Two cows were necropsied. Both had lymphohistiocytic inflammatory cell infiltrates with multinucleated giant cells in most lymph nodes with lymphadenomegaly and similar infiltrates in liver, kidney and spleen. Chronic suppurative dermatitis with orthokeratotic hyperkeratosis and lymphohistiocytic dermal infiltrates were found in addition to lymphohistiocytic to granulomatous infiltrates in the uterus. One cow with ataxia had lymphohistiocytic and granulomatous inflammatory lesions around spinal nerves and around the sciatic nerve. Lymphohistiocytic and granulomatous infiltrates were dominated by CD3-positive lymphocytes and Mac387-positive macrophages and multinucleated giant cells. CD79alpha-positive aggregates were a minor component of the infiltrates in the kidney. The lesions were consistent with lesions of citrus pulp toxicity reported in dairy cows and with the suspected hypersensitivity mechanism of the disease. Nerve involvement and ataxia were unique manifestations of this episode of toxicosis in beef cattle.

**Poster Number:** D-71

**Section:** Diagnostic Pathology  
**Keyword:** Wildlife

**INTESTINAL GIARDIASIS AND POST-TREATMENT ENTERITIS IN WILD-CAUGHT PRAIRIE DOGS**

National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA

Wild-caught prairie dogs are useful in biomedical research as models of some human infectious diseases and as sentinels of disease at the human-wildlife interface. We identified intestinal giardiasis in wild-
caught prairie dogs obtained by our laboratory for these purposes. Multiple colony animals presented for dramatic weight loss and soft stool, and necropsy of one of the most severely affected animals revealed mild chronic enterocolitis with mucosal surface-associated *Giardia* trophozoites. *Giardia duodenalis* was confirmed by electron microscopy and immunofluorescent and molecular assays. Colony-wide fecal sampling revealed a high prevalence of *Giardia* shedding, which led to initiation of fenbendazole treatment for all colony animals. Four animals died unexpectedly during or shortly after treatment, and necropsy revealed intestinal pathology in these animals ranging from hemorrhagic necrosis with bacterial colonization to villous blunting with epithelial dysplasia. Retrospective review showed that these four animals were among those with the lowest body weights in the colony at the time of treatment initiation. Because a standard dose of fenbendazole (50-100mg SID) was given to all animals regardless of weight, the leaner animals received a higher dose per body weight (100-200mg/kg SID). This higher dose was still within the expected margin of safety as reported for other species, and most animals in our colony experienced no overt adverse effects of treatment. However, given our findings and the paucity of information regarding fenbendazole use in prairie dogs, we propose that they may be more sensitive to fenbendazole toxicity than other species, especially if debilitated at the time of treatment.

**Poster Number:** D-72

**Section:** Diagnostic Pathology

**Keyword:** Wildlife

**INTRACEREBRAL MALIGNANT PLASMACYTOMA IN A MULE DEER (ODOCOILEUS HEMIONUS)**

C.S. Clancy¹, A. Roug², and A. J. Van Wettere¹

¹Utah State Veterinary Diagnostic Laboratory, School of Veterinary Medicine, Utah State University, Logan, UT, ²Utah Division of Wildlife Resources, Salt Lake City, UT

A wild, mature, gravid female mule deer (*Odocoileus hemionus*) was humanely euthanized by the Utah Division of Wildlife Resources due to neurological symptoms, including circling and incoordination. Grossly, a well-demarcated, 3x3x3 cm intracranial mass replaced the left olfactory bulb and adjacent rostral cerebrum. Histologically, a highly cellular, infiltrative and unencapsulated neoplastic mass of round cells with mild plasmacytoid features was observed. Neoplastic cells were positive for lambda light chain, occasionally positive for vimentin and negative for cytokeratin. A moderate number of Iba1, CD20, CD79a and CD3, and few kappa light chain positive cells were scattered throughout the neoplasm. Histology and immunohistochemistry results were diagnostic for a solitary extramedullary plasmacytoma. No evidence of disseminated disease was detected. While intracranial masses are rarely diagnosed in wildlife species exhibiting disturbances of behavior, they should be included in the differential diagnosis for potential causes of central nervous system disease. This is the first description of a primary intracranial plasmacytoma in mule deer.

**Poster Number:** D-73

**Section:** Diagnostic Pathology

**Keyword:** Primates
INTRANASAL OSTEOLIPOMA IN A CYNOMOLGUS MONKEY

O. Katsuta¹, T. Shibata¹, Y. Kuriki-Yamamoto¹, T. Mochizuki¹, M. Yoshimi¹, and T. Noto¹
¹Santen Pharmaceutical Co., Ltd., Nara, Japan

Intranasal lipoma of the nasal turbinates is an extremely rare tumor both in humans and animals. Recently we encountered a tumor in a cynomolgus monkey (Macaca fascicularis). The monkey, a 15-year-old male, was one of 6 animals treated as a human ocular disease model. The animal was clinically healthy except that both eyes had received a surgical operation. At the end of the study, the animal was euthanized by exsanguination from the axillary artery and abdominal aorta under deep anesthesia by intravenous injection of pentobarbital sodium.

At necropsy, the appearance of the nose was normal. After fixation, during the cross-section procedure on the nose, large bilateral masses were found at both tips of the middle turbinates. The longitudinal length of the right and left masses were 2 cm and 1.2 cm, respectively. Each mass had grown in the maxillary sinus on each side and occupied the cavity.

Decalcified samples were examined histopathologically. Then, both masses revealed benign lipomas of the turbinate bones. The tumors were composed of well-developed lipocytes, trabecular bones and a few blood vessels. We initially diagnosed the tumor as bilateral intranasal lipomas of the nasal turbinates. However, we could not differentiate this case from lipomatous hamartoma. The unique symmetrical proliferation, lack of border from the normal marrow, and the intact surrounding tissue might indicate a hamartoma.

This case was discussed at the 55th veterinary pathology slide forum at the 2nd meeting of the Japanese College of Veterinary Pathologists (JCVP) in March, 2015.

Poster Number: D-74

Section: Diagnostic Pathology
Keyword: Liver and Pancreas

LIVER FAILURE BY HYPOPLASIA OF THE GALLBLADDER IN A DOG

J.C. Osorio-Baños¹, J. Ocampo-López¹, and M.A. Servín-Trujillo¹
¹Servicio de Diagnóstico en Patología, Área Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de Hidalgo, Pachuca, Mexico

A 3 months old, male dog was admitted to the University Veterinary Hospital "UAEH" because it presented lethargy, anorexia, depression and urine red. On physical examination the patient presented hypothermia, dehydration and icteric in the mucous membranes, foot pads and skin. During the clinical approach samples were taken for blood count, blood chemistry and serology for Leptospira spp. Serum biochemistry showed high ALT, AST, GGT, ALP, Hyperbilirubinemia, hypertriglaceridiem and hypercholesterololemia. In tests liver failure and negative for Leptospira spp was observed. Euthanasia was requested by the owner. At necropsy the dog was markedly icteric, hepatomegaly it was observed with increased lobular pattern, rounded edges, friable to the touch, cut into the parenchyma a green stippling (cholestasis) was observed. The gallbladder was decreased in size, firm, white, rugged
appearance, the court had little viscous bile and bile duct atrophy was observed. Histologically, the hepatic cords were dissociated, the portal blood vessels are seen very crowded space, centrilocular degeneration and moderate congestion with evidence of severe intrahepatic cholestasis and cells had lost its core. Based on the above liver failure was caused by hypoplasia of the gallbladder was diagnosed. Hypoplasia of the gallbladder is an alteration of development of this structure, in which there are fewer cells than in normal conditions. Hypoplasia of the gallbladder is a rare condition, either in its unique form or in association with similar injuries in intra- and extrahepatic bile ducts.

**Poster Number:** D-75

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**MAST CELL RICH EPITHELIOTROPIC LYMPHOMA IN A CAT**

J. Ragsdale\(^1\), N. Takács\(^1\), and K. Kuroki\(^2\)  
\(^1\)New Mexico State University, Department of Agriculture Veterinary Diagnostic Services, Albuquerque, NM, \(^2\)University of Missouri College of Veterinary Medicine Veterinary Medical Diagnostic Laboratory, Columbia, MO

Multiple skin biopsies from a 9 years old, castrated male, Ragdoll cat were examined. The cat had intensely pruritic, red, excoriated and alopecic lesions that involved the skin of both the right and left lateral thorax. Microscopically, the skin contained a lichenoid infiltrate of round cells that extended around the hair follicles. The lichenoid infiltrate extended into the epidermis and hair follicle epithelium and formed Pautrier’s microabscesses. The round cells were 2.5 - 3 erythrocytes in diameter with small amounts of eosinophilic cytoplasm and round nuclei. There were large numbers of round cells within the lichenoid infiltrate that contained basophilic cytoplasmic granules. Differential diagnoses included epitheliotropic lymphoma, epitheliotropic mast cell tumor and epitheliotropic histiocytic proliferative diseases such as progressive dendritic cell histiocytosis. Metachromatic stains, Giemsa and Toluidine Blue, identified the cells with basophilic granules as mast cells, but the majority of the round cells, including those in the Pautrier’s microabscesses, did not contain metachromatic granules. Small numbers of these cells, including rare cells in the epidermis, stained positive for c-KIT and tryptase with immunohistochemistry (IHC). The majority of round cells, including those in the Pautrier’s microabscesses, were positive for CD3 using IHC confirming their T-lymphocyte origin. There were rare cells in the lichenoid infiltrate and in the epidermis that were positive for CD20 and CD79a using IHC. Thus, the lesions were diagnosed as mast cell rich epitheliotropic T-cell cutaneous lymphoma. The cat was euthanized two months after diagnosis due to progression of the disease to involve the entire skin.

**Poster Number:** D-76

**Section:** Diagnostic Pathology  
**Keyword:** Cardiovascular

**MICRO-CT OF FORMALIN FIXED CARDIAC TISSUE DETECTS MINERALIZATION IN GRMD CARDIOMYOPATHY**
Golden retriever muscular dystrophy is a genetically homologous model for the human disease, Duchenne muscular dystrophy (DMD). Affected animals develop a progressive cardiomyopathy with fibrosis and mineralization. Lesions initially appear in the posterobasal left ventricle and spread to include anastomosing bands of fibrosis throughout the left ventricle and septum with lesser involvement of the right ventricle and atria. The severity of changes varies between individuals and characterizing the extent of lesions is important for phenotypic stratification. We hypothesized that micro-CT of fixed tissue could be used to detect and map foci of mineralization in the hearts of GRMD dogs. Mineralization was detectable as hyperintense areas on micro-CT and confirmed by correlation of H&E and Von Kossa stains.

**Poster Number:** D-77

**Section:** Diagnostic Pathology

**Keyword:** Infectious Disease

**MYCOPLASMA HAEMOCANIS IN A SPLENECTOMIZED, THROMBOCYTOPENIC DOG WITH A RECENT HISTORY OF A COYOTE BITE**

L. Kelly¹, D. Volokhov², N. Gottdenker¹, A. Page-Karjian¹, and M. Camus¹

¹Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, ²Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, MD

*Mycoplasma haemocanis* is an epicellular bacteria with widespread, international distribution in domestic dogs, but typically only causes clinical disease in splenectomized, immunosuppressed, or compromised individuals. Organisms may be transmitted by the brown dog tick, oral administration of infected blood, and infected blood transfusions. *M. haemocanis* was visible in the peripheral blood of an 8 year old, male neutered, mixed breed dog for the first time 2.5 weeks after being bitten by a coyote (*Canis latrans*). The blood had been periodically monitored for nearly two years after the patient was diagnosed with hemangiosarcoma, and underwent splenectomy followed by metronomic chemotherapy. Chemotherapy had been discontinued 6 months prior to presentation. On presentation, the dog was markedly thrombocytopenic (platelet count = 38,000; RI = 235,000-634,000), but the complete blood count contained no other abnormalities. No other tick-borne diseases were diagnosed on a commercially available PCR panel (14 other pathogens), and the presence of *M. haemocanis* was confirmed with sequence analysis. Thrombocytopenia resolved with treatment with doxycycline and an anti-inflammatory dose of prednisone. Because the coyote was not available for sampling, the actual source of the infection is unconfirmed. However, the time course of infection makes transmission by the coyote bite extremely likely. As the range of the coyote increases in North America and becomes more urban and suburban, increasing coyote-dog interactions may alter the transmission risk of infectious, cross-species pathogens.

**Poster Number:** D-78
MYOPATHY OF THE TAIL MUSCLES IN BEAGLE DOGS USED IN PRECLINICAL TOXICITY STUDIES

M. Abdi, H. White, and J. Boxall

Two male and two female beagle dogs aged 11 to 20 months, which were used in preclinical toxicity studies, were presented with altered tail carriage following jacketing for non-invasive electrocardiogram monitoring (EMKA Technologies, France). The proximal one third of the tail was held in a horizontal plane with the distal two-thirds hanging ventrally with reduced tone and tenderness on palpation. A diagnosis of “limber tail” was made based on the clinical signs. The dogs were necropsied at the end of the studies. No macroscopic changes were observed in the tail muscles. Microscopically, moderate to marked degeneration/necrosis involving one or more muscle bundles was seen in three of the four animals. There were no associated clinical pathology changes. Spontaneous acute myopathy of the tail has been reported in a number of breeds of working dogs, and was considered to be the consequence of prolonged exposure to cold water, vigorous exercise or prolonged cage transportation. Although the dogs in the present case were not exposed to cold water or extreme activities, dogs wearing jackets have been observed to shake vigorously following the removal of the jacket. There is no evidence of jackets used for non-invasive electrocardiogram monitoring resulting in myopathy of the tail, but it is postulated that repeated vigorous shaking after their removal could be a contributory factor in the development of the lesion.

NATIONAL REGISTRY OF FORENSIC VETERINARY PATHOLOGY: IT MIGHT BE A WORTHWHILE EFFORT!

A. Wünschmann¹, S. P. McDonough², J. Gerdin², B.J. McEwen³, and J.W. Brooks⁴
¹Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, MN, USA, ²Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA, ³University of Guelph, Animal Health Laboratory, Ontario, Canada, ⁴Pennsylvania State University, Animal Diagnostic Laboratory, University Park, PA, USA

Veterinarians, including veterinary pathologists, play a critical role in holding perpetrators of animal abuse and neglect accountable on one hand and on the other hand protecting individuals from unjust prosecution by providing evidence and helping members of the court system understand the pathologic findings and their legal implications. Although reliable data regarding the incidence of animal abuse and neglect are unavailable, anecdotal observation suggests that diagnostic veterinary pathologists are regularly confronted with suspect cases. These cases frequently are complex and questions are being posed by the prosecutors that are outside those of routine diagnostic cases. Although forensic pathology experience exists within the American College of Veterinary Pathologists (ACVP), tapping into this
resource is difficult. We recommend the ACVP host a registry of forensic pathology. This registry would collate cases in which pathology reports were involved in the prosecution of animal abuse and neglect. A template would be developed that would be filled in by case contributors. The information could include the nature of the case, the science of the veterinary pathology that was applied, applicability and availability of ancillary testing, the nature and content of the interrogation by the attorneys, and the outcome of the case. Not only would this registry serve as a valuable resource for veterinary pathologists when examining similar cases but also as a medium to collect quantifiable data regarding animal abuse and neglect.

**Poster Number:** D-80

**Section:** Diagnostic Pathology

**Keyword:** Neoplasia

**OLFATORY NEUROBLASTOMA AND NEUROENDOCRINE CARCINOMA IN TWO DOGS. IMMUNOHISTOCHEMISTRY DIAGNOSTIC CRITERIA - CASE REPORT**

M.D. Ronderos¹, N.A. Cortés¹, B. Doncel², P. Barato¹²³, and J.C. Ospina³

¹Corporación Patología Veterinaria (CORPAVET), Veterinary Anatomic Pathology Division, Bogotá D.C., Colombia; ²Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, National University of Colombia, Bogotá D.C., Colombia; ³Instituto Colombiano Agropecuario, ICA, Veterinary Pathology Division, Bogotá D.C., Colombia

Samples from two adult male canines with history of epistaxis, nasal discharge, frontal region pain, chronic sinusitis and presence of a mass in the nasal cavity with invasion and destruction of nasal bones were submitted to CORPAVET. Histopathologically, both cases showed a non-encapsulated mass, composed of neoplastic cells arranged in solid clusters, sheets, and lobules that were separated by delicate fibrovascular connective tissue with formation of rosettes or pseudorosettes. Grimelius stain and immunohistochemistry to protein gene product 9.5 (PGP 9.5; neuron-specific protein), neuronal cell adhesion molecule (CD56), S-100 protein, neuron-specific enolase (NSE), neurofilament protein and cytokeratins (CK AE1 / AE3) were performed. One case was immunochemical (NSE, CD56, PGP 9.5) and grimelius positive for neuronal and neurofilament components and negative to epithelial antigens (CK AE1/AE3) indicating neuroectodermal origin with mainly neuronal differentiation; the final diagnosis was olfactory neuroblastoma. In contrast, the second case was immunopositive for epithelial antigens (CK AE1/AE3) and neuronal markers (NSE) while was negative for the other antigens, defining as neuroectodermal origin with mainly epithelial differentiation; this case was diagnosed as neuroendocrine carcinoma. Both neoplasms are considered as difficult diagnose for their histopathological similarities for that reason is important establish a useful panel of immunohistochemistry to determine the final diagnosis.

**Poster Number:** D-81

**Section:** Diagnostic Pathology

**Keyword:** Infectious Disease
PARVOVIRAL MYOCARDITIS IN A LITTER OF PUPPIES

K. Atkinson¹, J. Ragsdale², and N. Takács²
¹Petroglyph Animal Hospital, Albuquerque, NM, ²New Mexico State University Department of Agriculture Veterinary Diagnostic Services, Albuquerque, NM

Four of six puppies in a mixed-breed litter died suddenly between the ages of 2.5 and 3 weeks. The dam had died of an unknown cause one week after giving birth. After death of the dam, the puppies were doing well until they were reported to develop acute dyspnea, cyanotic mucous membranes and collapsed and died within minutes. One puppy was necropsied. Grossly, the lungs were heavy on palpation and pericardial effusion was noted. The heart and lungs were submitted for histopathology. Microscopically, the myocardium contained multifocal infiltrates of mostly lymphocytes and macrophages with rare plasma cells. The inflammatory infiltrates were often associated with small numbers of necrotic cardiac myofibers and myofiber loss. There were rare cardiac myofibers containing basophilic intranuclear inclusion bodies. Multiple cardiac myofibers were positive for parvoviral antigen via immunohistochemistry confirming the puppy died of parvoviral myocarditis. The microscopic findings in the lungs were consistent with chronic passive congestion. The two remaining puppies in the litter died shortly after the submission of the puppy for necropsy. Parvoviral myocarditis is rarely confirmed presently due to widespread vaccination and natural exposure of reproductive age female dogs that pass maternal antibodies to susceptible neonatal puppies. Generally, the myocardial form occurs in puppies that have not received maternal antibody and are infected with parvovirus before six to eight weeks of age.

Poster Number: D-82

Section: Diagnostic Pathology
Keyword: Neoplasia

PATHOLOGIC FEATURES OF A NASAL ADENOCARCINOMA IN A CYNOMOLGUS MACAQUE (MACACA FASCICULARIS)

M. Novilla¹, M. Cottingham², E. Mendoza², J. Bernal², K. Breyer², and S. Jacobson¹
¹Shin Nippon Biomedical Laboratories (SNBL) USA, Ltd, Everett, WA, ²Shin Nippon Biomedical Laboratories (SNBL) USA SRC, Alice, TX

An 8 year old intact female cynomolgus monkey (Macaca fascicularis) undergoing treatment for bilateral conjunctivitis was euthanized when her condition progressed to severe swelling across the bridge of the nose and lateral deviation of the left eye. Skull radiographs showed a fractured nasal bone with lysis of the nasal bridge. Surgical exploration revealed a large hollowed area in the sinonasal cavity, at least 5 cm deep, surrounded by abnormal proliferative tissue. At necropsy, a large, irregularly shaped, soft, friable, proliferative mass with indistinct margins, occupied the left sinonasal cavity, penetrating the cribriform plate and displacing the inner wall of the orbit outward. Histologically, the tumor mass arose from the respiratory epithelium of the nasal septum and consisted of large papillary tubular outgrowths arranged in lobules with focal areas of necrosis, separated by fibrovascular stroma with mixed inflammatory cell infiltrates. Neoplastic cells had mild mitotic activity. Immunohistochemical staining for chromogranin and synaptophysin, biomarkers for neuroendocrine differentiation, were
negative. Therefore, a final diagnosis of nasal papillary tubular adenocarcinoma was given. To the best of our knowledge, this is first report of a nasal adenocarcinoma in a cynomologus (*Macaca fascicularis*) monkey.

**Poster Number:** D-83

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

**PATHOLOGICAL, IMMUNOHISTOCHEMICAL, AND MOLECULAR CONFIRMATION OF SENECAVIRUS A IN PIGLETS FROM SOUTHERN BRAZIL**

S.A. Headley¹, R.A. Leme², T.E.S. Oliveira², B.K. Alcântara², L.A. Freitas², A.F. Alfieri², M. Yang³, and A.A. Alfieri²  
¹Laboratory of Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Paraná, Brazil; ²Laboratory of Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Paraná, Brazil; ³National Center for Foreign Animal Disease, Winnipeg, Canada

During the last few months, there were reports of an undiagnosed vesicular disease affecting pig herds from several geographical regions of Brazil. This study investigated the cause of the vesicular lesions in these piglets. Four 1-2 day-old piglets from two pig farms located in different cities of southern Brazil were submitted for diagnostic evaluations. Significant gross pathological findings included diphtheric glossitis (n=4) and ulcerative lesions at the coronary band (n=2). Histopathology revealed necrotizing glossitis with ballooning degeneration of epithelial cells (n=4), interstitial pneumonia (n=4), myocarditis (n=3), and necrotizing encephalitis (n=3). An immunohistochemical analysis using a SVV-specific monoclonal antibody revealed positive immunostaining at the degenerated epithelium of the ulcerative lesions of the tongue in all piglets. RT-PCR assays were used to amplify specific amplicons of viral agents associated with vesicular diseases: SVV, foot and mouth disease, vesicular stomatitis, swine vesicular disease, *Teschovirus A*, *Sapelovirus A*, *Enterovirus G*, porcine circovirus-2 and porcine parvovirus. Amplicons of the SVV were amplified from multiple tissues of all piglets; all other RT-PCR assays were negative. Sequence analyses and virus isolation confirmed the SVV RT-PCR assay; phylogenetic evaluation revealed that the isolates from Brazil clustered with similar strains of SVV identified in North America. The pathological, immunohistochemical, and molecular findings confirmed the participation of SVV in the lesions observed in these piglets, other pathogens associated with vesicular lesions of swine were excluded due to the negative results of the RT-PCR assays. Consequently, these results extend the geographical distribution of SVV to include Brazil.

**Poster Number:** D-84

**Section:** Diagnostic Pathology  
**Keyword:** Avian
PATHOLOGY AND DIAGNOSTIC CRITERIA FOR NECROTIC ENTERITIS OF CHICKENS

M. Ronderos¹, D. Gornatti², H. Sivaprasad³, and F.A. Uzal³
¹Program of Veterinary Medicine, La Salle University, Colombia, ²Faculty of Veterinary Sciences, National University of La Plata, Argentina, ³California Animal Health and Food Safety Laboratory System, School of Medicine, UC Davis, Davis, California, USA

Diagnosis of necrotic enteritis (NE) produced by Clostridium perfringens in poultry can be challenging, mainly because there are overlapping lesions between NE and coccidiosis, the main predisposing factor for NE. In addition, C. perfringens is usually found in the intestine of normal chickens, which renders cultural procedures of little diagnostic help. We compared gross and microscopic lesions in chickens with NE (n=128), NE and coccidian co-infection (NE+coccidian n=223) and coccidiosis alone (n=61). 73.43% of chickens with NE, 97.75% of chickens with NE+coccidian and 73.77% of chickens with coccidiosis had gross lesions in at least one portion of the intestine. A small percentage of birds with NE+coccidian had also gross lesions in cecum and liver (5.87% and 9.41%, respectively). Necrosis of the intestinal mucosa was observed microscopically in all chickens from the three groups. The necrosis extended to the submucosa and muscularis in 0.8% of chickens with NE, in 21.5% of the chickens with NE+coccidian and in 34.4% of chickens with coccidiosis alone. Large numbers of Gram positive rods, usually in clusters, were seen associated with the necrotic lesions in cases of NE and NE+coccidian, but not in cases of coccidiosis alone. Coccidian organisms were observed in cases of NE+coccidian and coccidiosis alone. Lesions were more severe in cases of NE+coccidian than in the chickens from the other two groups. The subtle differences in gross and microscopic lesions between some cases of NE+coccidian and coccidiosis alone, make the differentiation of the two conditions challenging.

Poster Number: D-85

Section: Diagnostic Pathology
Keyword: Neoplasia

PERITONEAL Rhabdomyosarcoma in a Cat

L.Á. Garcia-Camacho¹, M. López-Montaño, C. Cedillo-Pelaez², F.J. Vazquez-Garcia³, E. Farfán-Morales³, and I.C. Rangel-Rodriguez¹
¹Departamento de Ciencias Biológicas, Facultad de Estudios Superiores Cuautitlán, UNAM, ²Laboratorio de Inmunología Experimental, Torre de Investigación, Instituto Nacional de Pediatría, ³Departamento de Anatomía Patológica, Instituto Nacional de Pediatría, Mexico

A 12 year-old non-spayed female cat with complain of hyporexia and abdominal distention was presented to teaching hospital. The ultrasound revealed pyometra and a solid mass throughout abdominal cavity. At exploratory laparotomy, pyometra was confirmed and multiple, white to tan, variable-size nodules were found on both parietal and visceral peritoneum, extending from omentum to retroperitoneum. The euthanasia was performed at the owner’s request. After uterus and abdominal mass removal, look-alike multiple nodules were observed on diaphragm. Microscopically, non-circumscribed tumors composed of highly pleomorphic cells arranged in bundles surrounded by fine fibro-vascular tissue. The tumor cells are both round to irregular with cytoplasm fully-vacuolated,
revealing eosinophilic strands (spider-cells) and multinucleated giant cells with intensely eosinophilic cytoplasm and perinuclear vacuoles. The nuclei are round to oval with vesicular to coarse stippled chromatin and 1-3 prominent nucleoli. Karyomegaly, anisokariosis, and row-arrangement of nuclei are common. The mitotic index is high (8-10 bizarre mitosis per hfp). At trichrome stain, the tumor cell cytoplasm is red and displays cross-striations. The IHC revealed that tumor cells are negative to cytokeratin AE1/AE3 and diffusely and strongly reactive to vimentin. Approximately 70% of multinucleated and splinde tumor cells are strongly positive for Mio2 but only around 5% of pleomorphic cells stained for this marker. The tumor cells stained negative for smooth muscle actin. Taken altogether, a final diagnosis of rhabdomyosarcoma (embryonal/pleomorphic) was made, most likely arising from diaphragm since there was no sarcoma elsewhere. Given its location and extension, the present case is extremely rare.

**Poster Number:** D-86

**Section:** Diagnostic Pathology  
**Keyword:** Hematopoietic System

**PERSISTENT THROMBOCYTOSIS IN A DOG**

D. Hoffman¹, N. Hoepp², and A. Royal¹  
¹Veterinary Medical Diagnostic Laboratory, University of Missouri, College of Veterinary Medicine, Columbia, MO, ²IDEXX Laboratories, Inc., Westbrook, ME

An 8-year old spayed female Brussels Griffon was presumptively diagnosed with chronic myeloproliferative neoplasia based on splenic mass biopsy, bone marrow, and CBC results. Marked thrombocytosis persisted for >1 year after splenectomy. During follow-up, pituitary dependent hyperadrenocorticism (PDH) was diagnosed and managed. While multiple potential causes for secondary/reactive thrombocytosis existed in this case, a myeloproliferative neoplasm (MPN) was considered most likely based on the extent of bone marrow megakaryocytic hyperplasia, duration and degree of persistent thrombocytosis, and lack of platelet concentration change during treatment for PDH. Specific chronic MPNs which cause thrombocytosis include essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF), however this patient lacked the erythrocytosis expected with PV, as well as the megakaryocytic dysplasia and marrow fibrosis expected with PMF. All chronic MPNs with associated thrombocytosis are rare in dogs and difficult to definitively diagnose, as molecular diagnostics are limited in veterinary hematology. Isolation of a JAK2 V617F mutation in humans is diagnostic for PV (>95% of cases), however this mutation is also present in approximately 60% of ET and PMF cases. Initial assessment for mutations in JAK2 are pending in this case. While these mutations have not been firmly characterized in dogs, identifying JAK2 mutations in canine patients could prove helpful in diagnosing/categorizing MPNs and may lead to insight on treatment.

**Poster Number:** D-87

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia
PLEOMORPHIC ADENOMA OF THE SUBMANDIBULAR SALIVARY GLAND IN A HORSE

S. Diab
California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, CA

Pleomorphic adenoma is the most common, benign, salivary gland tumor of humans, but is rarely diagnosed in animals. We describe a submandibular neoplasm in a 28-year-old, female Paint horse that was histologically consistent with pleomorphic adenoma of the salivary gland of humans. Twenty small, unencapsulated, tan or dark red pieces of tissue were examined. Histologically, there was a variably dense subcutaneous population of neoplastic epithelial cells (epithelial component) supported by abundant myxoid/mucoid stroma populated with moderate numbers of spindloid cells (myoepithelial component). Some sections showed predominance of the epithelial component, whereas others were predominantly stroma. Neoplastic epithelial cells were closely packed, arranged in duct-like structures, nests, or sheets, were cuboidal or basaloid type and had moderate amount of poorly distinct, basophilic cytoplasm. Nuclei were round to oval, hyperchromatic, with finely stippled or condensed chromatin and had one or two nucleoli. Anisocytosis and anisokaryosis was mild. Mitoses averaged 1 per 10 hpf. Occasionally, thin bony trabeculae were entrapped and focally invaded by the neoplasm. The epithelial component was strongly positive for pancytokeratin and negative for vimentin, smooth muscle actin, S-100 and GFAP immunohistochemistry. The myoepithelial component was strongly positive for vimentin and smooth muscle actin, weakly positive for S-100 and negative for pancytokeratin and GFAP. Collectively, these findings were compatible with a diagnosis of pleomorphic adenoma with osseous differentiation of the salivary gland of humans. Although usually a benign tumor of people, no follow-up information was available in this horse, for which the post-surgical clinical behaviour of this tumor is unknown.

Poster Number: D-88

Section: Diagnostic Pathology
Keyword: Cardiovascular

PLEXIFORM PULMONARY ARTERIOPATHY IN A DOG

S. Kumar1 and A.J. Cooley2
1National Animal Disease Center, USDA-ARS, Ames, IA, 2College of Veterinary Medicine, Mississippi State University, Starkville, MS

An 11-year-old Husky was presented for necropsy with a history of dyspnea and multiple episodes of collapse. At necropsy, the lungs were bilaterally hyper-inflated, pink to reddish-purple, with a focal 2 cm long, firm, tortuous cordlike lesion on the right-dorsal caudal lung lobe. The right atrium and ventricle were dilated, and the main pulmonary artery was partially thrombosed. The liver was diffusely congested. Both kidneys were small, and had irregular dark depressions on the cortex. Histopathology revealed profound changes in pulmonary arteries. Multiple arterioles had narrow lumina, thick tunica media, and marked irregular hypertrophy of tunica intima. Often, the arterioles appeared in clusters with multiple profiles suggesting tortuosity. Peripheral arterioles had subdivision of the lumen into
multiple irregular channels. Multifocal arteries had plexiform lesions evidenced by small irregular vascular spaces with peripheral intact tunica media. The liver had severe centrilobular congestion. Both kidneys had changes consistent with end stage kidneys. Based upon the gross and histopathological characteristics, this was diagnosed as a case of plexiform pulmonary arteriopathy resulting in primary pulmonary hypertension, right heart failure, passive congestion, and vascular thrombosis. No evidence of heartworm infection was found. Plexiform pulmonary arteriopathy is rarely reported in veterinary literature. These lesions are called plexiform because of the development of complex vascular structures in which endothelial cells predominate sprouting abruptly in pulmonary arterioles. The plexogenic pulmonary arteriopathy is considered as pathologic hallmark of primary pulmonary hypertension. The cause is not yet identified, although genetics and aberrant activation of VEGF/VEGFR pathway have been implicated.

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**Poster Number:** D-89

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

**PSEUDOTUBERCULOSIS: AN EASILY MISSED RESPIRATORY PATHOGEN IN IMMUNOSUPPRESSED RATS**

T-Y Chen¹, Y-H Chang¹, Y-L Chen¹, and K-H Lee¹  
¹National Laboratory Animal Center, National Applied Research Laboratories, Taiwan

*Corynebacterium pseudodiphtheriticum* has uncommonly been reported to occur in laboratory rats and mice, but *C kutscheri* is a common bacterium isolated from the oral cavity of healthy mice and rats. This case report describes the rare finding of pseudotuberculosis in Lewis rats following coronary artery ligation and immunosuppressive therapy. The rats appeared weight loss, ruffled fur, dyspnea, chromodacryorrhea, hunched posture and lameness. Gross findings included multifocal randomly distributed abscesses, abscesses in the liver and kidney, and multiple abscess around surgical suture area. On microscopic examination, the lesions were necro suppurative pneumonia, caseous necrosis in the these suppuratives lesion and multiple abscesses with pyelonephritis. The histopathology results are related to gross findings. The Gram stain of the swab from the lung was positive. A heavy pure growth of Gram-positive bacillus was identified as *Corynebacterium kutscheri* by API system. Infection with this agent is usually subclinical in rats and mice and results in disease expressions only after severe immunosuppression.

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**Poster Number:** D-90

**Section:** Diagnostic Pathology  
**Keyword:** Alimentary

**SEPTICEMIA WITH NECROTIZING ENTERITIS BY LISTERIA MONOCYTOGENES IN A BOVINE CALF**

H. Taylor¹, W. Fales¹², M. Calcutt², T. Reilly¹², I. Ganjam¹, J. Bowman¹, and D.Y. Kim¹²
Listeria monocytogenes is a gram positive, facultative anaerobic bacteria, which often causes meningoencephalitis in ruminants. Septicemic listeriosis has been described in reindeer calves, horses, mice, human infants, and calves but reports of necrotizing enteritis have only been found in human infants and experimentally in mice. This report describes septicemia with necrotizing enteritis caused by L. monocytogenes in a 6-day old bovine calf. A local veterinarian submitted fresh and formalin-fixed tissues of a 6-day old Angus calf to the University of Missouri Veterinary Medical Diagnostic Laboratory. The clinical history was a 6 day old calf became lethargic and died. Histologic findings consisted of severe necrotizing enteritis, multifocal hepatic microabscesses, and bacterial emboli in the lungs. L. monocytogenes was isolated from the liver, lung, and small intestine and immunohistochemistry confirmed that the lesions were directly associated with listeriosis. Exact source of infection was not determined but contaminated milk was strongly suspected as the cause. This case report describes a septicemia with necrotizing enteritis by L. monocytogenes in a 6-day old calf, which is similar to the neonatal septicemia of human infants.

**Poster Number:** D-91

**Section:** Diagnostic Pathology  
**Keyword:** General

**SODA AND SNACKS: APPLYING VETERINARY FORENSIC TECHNIQUES TO FOOD CONTAMINATION**

A.B. Schaffer-White\(^1\) and R.E. Allavena\(^1\)  
\(^1\)School of Veterinary Science, The University of Queensland, Gatton, Australia

Three cases of alleged rodent contaminated food and soda were presented to the veterinary pathology department at UQSVS. Two cases involved intact or partial rodent cadavers that were discovered inside bags of newly purchased product and the manufacturer requested assistance. Another case involved a mouse cadaver inside a freshly opened can of cola-type soda, which was a matter of a legal case against the manufacturer. Veterinary pathologists were called in to determine if the presence murine contamination had occurred at the point of product manufacture. Autopsy examinations were conducted on murine cadavers and body parts to determine cause of death. In addition examination of product, investigation of product manufacturing practices and the effect of food substrates on murine cadaver preservation were undertaken.

In mixed nuts, dry roasting and salting a mouse both preserves the external appearance and mummifies internal organs. Examination of product for mouse predation or faeces can indicate if a mouse is bagged alive verses a cadaveric contaminant of an initial component. The canning to consumption interval for the soda mouse was approximately 1 month, but the carcass was very well preserved. In order to assess the verity of the consumers’ claim that the soda was contaminated at the factory, a pilot study into the effects of cola drink on the preservation of mouse tissues was undertaken. Over 6 weeks, ethically obtained mouse cadavers were submerged in the same brand cola drink at 4C and examined at regular intervals. Cola was determined to be a remarkably good murine tissue preservative.
SURFACTANT PROTEIN-A AS AN IMMUNOHISTOCHEMICAL MARKER OF CANINE PRIMARY PULMONARY CARCINOMAS

J.A. Beck1,2, M.A. Miller1, C.B. Frank3, D.M. DuSold1, and J.A. Ramos-Vara1
1Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, 2NIH CBSTP, National Cancer Institute, Bethesda, MD, 3Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO

Immunohistochemistry for thyroid transcription factor-1 (TTF-1), napsin A, and surfactant protein A (SP-A) is used in the diagnosis of human non-small-cell lung cancer. TTF-1 and napsin A are highly sensitive markers for canine primary pulmonary tumors; however, less is understood about the utility of SP-A in veterinary species. The objective of this study was to evaluate the sensitivity of SP-A in canine pulmonary carcinomas and compare it to that of TTF-1 and napsin A. Sixty-seven formalin-fixed, paraffin-embedded, canine pulmonary carcinomas were examined with antibodies to SP-A (goat polyclonal, sc-7700, Santa Cruz Biotechnology), TTF-1 (mouse monoclonal, 8G7G3/1, Dakocytomation), and napsin A (rabbit monoclonal, ACI 3043, Biocare). Immunoreactivity was scored by the percent positive neoplastic cells as 0 (no reactivity), 1 (1-15%), 2 (16-50%), or 3 (>50%), and by intensity as 0 (no immunoreactivity), 1 (low), 2 (moderate), or 3 (high). TTF-1, napsin A, and SP-A reactivity was detected in 97%, 97% and 99% of canine pulmonary carcinomas, respectively. Mean intensity of immunoreactivity for TTF-1, napsin A, and SP-A was 2.1, 1.8, and 2.0, respectively. Immunoreactivity varied with the histologic pattern with expression in a greater percentage of cells in acinar and papillary patterns compared to tumors with squamous differentiation. Based on these findings, SP-A is considered a highly sensitive immunohistochemical marker of canine primary pulmonary carcinomas with comparable performance to that of TTF-1 or napsin A. However, SP-A immunoreactivity can be difficult to distinguish from extracellular surfactant in alveolar spaces or coating the apical border of pneumocytes.

SYSTEMIC CORONAVIRUS-ASSOCIATED DISEASE CAUSED BY “FERRET ENTERIC CORONAVIRUS” IN A FERRET (MUSTELA PUTORIUS FURO)

H. Kondo1, H. Toge2, S. Tsuruno2, Y. Une3, S. Minami4, and K. Maeda4
1Synergy Animal General Hospital, Saitama, Japan, 2Exotic Pet Clinic, Kanagawa, Japan, 3Azabu University, Kanagawa, Japan, 4Yamaguchi University, Yamaguchi, Japan

In Japan, the occurrence of systemic coronavirus-associated disease resembling feline infectious peritonitis (FIP) and epizootic catarrhal enteritis has been sporadically reported. A 1.5-year-old, spayed female ferret weighing 580g presented with anorexia and diarrhea. Hematologic evaluation revealed
leukocytosis and hyperglobulinemia. By ultrasonography, splenomegaly and marked swelling of the mesenteric lymph node was detected. When exploratory laparotomy was performed, large amounts of ascites, swelling of the mesenteric lymph nodes and splenomegaly were observed. Cytologically, the ascites was composed of numerous neutrophils and macrophages with fewer numbers of lymphocytes and plasma cells. On the omentum, mesentery, and serosal surfaces of the intestine, there were numerous, tan nodules measuring 1-2mm in diameter. Histologically, multifocal to coalescing, granulomatous to pyogranulomatous inflammation was observed in the lesions. Special stains ruled out mycobacterial and fungal infections. By immunohistochemical staining using anti-FIP antibody, coronaviral antigen was detected in the granulomatous foci of the spleen. For the definitive diagnosis, the formalin-fixed splenic tissue was proceeded to the molecular method. We detected ferret enteric coronavirus (FRECV) RNA from the splenic tissue using genotype-specific reverse transcription polymerase chain reaction, and ferret systemic coronavirus (FRSCV) RNA was not amplified. The reason why the systemic disease was caused by FRECV in this case is still unclear, however, two possibilities are considered; 1) FRECV may potentially share similar characteristics and virulence with those of FRSCV, and 2) mutation among ferret coronaviruses is possible. To the authors’ best knowledge, this is the first report of ferret systemic and severe disease resembling FIP caused by FRECV.

**Poster Number:** D-94

**Section:** Diagnostic Pathology  
**Keyword:** Wildlife

**SYSTEMIC HISTOPLASMOSIS IN AN ADULT PORCUPINE (ERETHIZON DORSATUM)**

A. Hattel¹, R. Graboski², and J. Brown³  
¹ Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA,  
² Centre Wildlife Care, Lemont, PA, ³ Pennsylvania Game Commission, University Park, PA

*Histoplasma capsulatum* is a dimorphic fungus commonly found in soil that contains large amounts of bird and bat fecal material. The organism has a worldwide distribution including the Americas, Africa, Asia and Australia. In the United States, the organism is most prevalent in the Ohio and Mississippi river valleys and can produce respiratory and systemic lesions in a variety of animal species, including humans. This report characterizes systemic *Histoplasma capsulatum* infection in an adult porcupine (*Erethizon dorsatum*) with severe brainstem and pulmonary pathology. Microscopic lesions in the brainstem and lung included severe pyogranulomatous inflammation and necrosis intermixed with numerous *Histoplasma capsulatum* organisms. Polymerase chain reaction (PCR) procedures performed on formalin fixed tissue confirmed the presence of *Histoplasma capsulatum* within the brainstem.

**Poster Number:** D-95

**Section:** Diagnostic Pathology  
**Keyword:** Infectious Disease

**TRICHOMEONADS AND BRACHYSPIRA MURDOCHII INFECTION IN PIGS WITH COLITIS**
F. Giannitti, C. Gebhart, J. Sarradell, K. Sverlow, and M. Culhane

Necropsies of 5 pigs, 10-13 weeks of age, with diarrhea and weight loss, were performed at two swine farms, each housing >15,000 growing pigs. Tissue samples including colon were submitted to the UMN-VDL between 12/19/2014 and 1/9/2015. Histologic examination of the colon in all cases revealed moderate (cases 1-4) to mild (case 5) proliferative colitis with intraliesional trichomonads. Brachyspira murdochii was isolated from the colon and detected by immunohistochemistry in cases 3-5, but not in cases 1-2. Positive immunoreactivity for Trichomonas spp. in sections of colon was observed in cases 1-2 by immunohistochemistry, performed at UC-Davis. Trichomonas spp. were present in the lumen of dilated and hyperplastic mucosal glands, invading the lamina propria, and rarely in the cytoplasm of goblet cells and enterocytes. Trichomonads were also found within the cytoplasm of mucosal gland and proprial macrophages (phagocytosis), suggesting elicitation of an innate immune response.

Common causes of colitis in pigs also include Lawsonia intracellularis and Salmonella enterica; however, they were not detected in these 5 index cases. Interestingly, L. intracellularis and/or Salmonella spp. were detected in the colons of several pigs submitted subsequently from both farms (data not shown).

Lesions in these pigs were consistent with an infectious colitis. Trichomonads infection with or without Brachyspira murdochii co-infection was diagnosed in all cases. Both agents have been considered facultative enteric pathogens in swine. Whether this co-infection was the inciting factor for the colitis is controversial. We speculate that the severity of colonic lesions induced is greater when both pathogens are present.

**Poster Number:** D-96

**Section:** Diagnostic Pathology  
**Keyword:** Alimentary

**UNDIFFERENTIATED CARCINOMA IN THE MANDIBLE OF A HOLSTEIN COW**

N. Horiuchi\(^1\), S. Kayo\(^1\), K. Yoshimoto\(^2\), H. Inokuma\(^3\), and Y. Kobayashi\(^1\)  
\(^1\)Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, \(^2\)Tokachi Agricultural Mutual Aid Association, Obihiro, Japan

A 4-year-8-month-old Holstein cow was admitted for diagnosis of a large swelling on the left mandible. Core needle biopsy of the mass revealed atypical round cells with moderate anisokaryosis and numerous mitotic figures, leading to a suspected diagnosis of lymphoma. However, haematologic parameters proved negative of lymphocytosis and an antibody test for BLV was also negative. On necropsy, a moderately firm and poorly demarcated subcutaneous mass measuring 40 x 30 x 28 cm was present in the left mandibular region. Due to the large mass, the trachea and esophagus were displaced to the right and the laryngopharynx was stenosed. Neoplastic masses were also found in the paratracheal region. Histologically, all tumour masses were composed of solid growth of small round cells with occasional glandular and rosette-like structures. Differential diagnosis at this point included lymphoma, undifferentiated carcinoma, neuroblastoma and primitive neuroectodermal tumour (PNET). Immunohistochemical staining of the neoplastic cells revealed that the epithelioid cells were
immunopositive for cytokeratin AE1/AE3 and negative for vimentin, while part of the small round cells expressed both cytokeratin AE1/AE3 and vimentin. All neoplastic cells were negative for CD3, CD20, HLA-DR, P63, α smooth muscle actin, desmin, S100, neuron specific enolase, neurofilament, glial fibrillary acid protein, synaptophysin and chromogranin A. The final histological diagnosis was undifferentiated carcinoma. On the basis of the site of tumour mass, immunopositivity toward cytokeratin and vimentin, and lack of recognisable differentiation except for epithelial structures suggest the tumour being of salivary gland origin.

**Poster Number:** D-97

**Section:** Diagnostic Pathology  
**Keyword:** Urinary System

**UNILATERAL URETERAL FIBROEPITHELIAL POLYP IN A DOG**

A. Berrocal† and W. Moreno†  
†Histopathovet, Heredia, Costa Rica

Primary ureteral neoplasias have been rarely described in human and domestic animals. In dogs only 15 documented ureteral neoplasias have been reported, six of them as a fibroepithelial polyps.

A 9-year-old, spayed female French poodle was presented with history of intermittent hematuria. At physical examination a distended abdomen was observed. At palpation a non-painful mass of 7.0-8.0 cms was found in the right caudal abdomen. All other parameters were normal. The mass was also observed by ultrasound. A laparotomy was performed to remove the mass that was attached to the right kidney and it was submitted for histopathological examination.

Grossly: the kidney size was 10.0 cms. At cut surface it was filled with brown fluid and corticomedullary atrophy. Attached to the kidney was a 22.0 cm long, dilated thin walled ureter ending with a 10.0 cm irregular mass partially attach to the mucosa obstructing the lumen.

Histopathology: The kidney showed a complete atrophy of the pelvis and medulla, with a thin fibrotic cortex. The ureteral mass consisted of papillary fronds lined by transitional epithelium with vacuolization. Internally the fronds were composed of fibrovascular tissue. The final diagnosis was a ureteral fibroepithelial polyp with secondary hydroureter and hydrourephrosis.

Ureteral fibroepithelial polyps are rare in veterinary medicine as well as in humans. In fact this is the seventh case reported. Despite been a benign tumor clinically it is associated with urinary incontinence, infections, hematuria, hydronephrosis and hydroureter as it was observed in this patient.

**Poster Number:** D-98

**Section:** Diagnostic Pathology  
**Keyword:** Neoplasia

**VASCULAR LEIOMYOSARCOMA IN A DOG**
L.A. García-Camacho¹, C. Cedillo-Pelaez², F.J. Vázquez-García³, E. Farfan-Morales³, and I.C. Rangel-Rodriguez¹
¹Departamento de Ciencias Biolórgicas, Facultad de Estudios Superiores Cuautitlán, UNAM, ²Laboratorio de Inmunología Experimental, Torre de Investigación, Instituto Nacional de Pediatría, ³Departamento de Anatomía Patológica, Instituto Nacional de Pediatría

A mixed-breed 3-year-old male dog with a tumor mass on right anterior limb between scapulae and axilla, was presented to teaching hospital. Microscopically, the tumor was composed of markedly pleomorphic spindle cells disposed both concentrically on vascular walls which are moderately dilated by erythrocytes or less commonly in solid areas in which vascular structures are scarce. The tumor cells display marked anisocytosis and anisokariosis, scant eosinophilic cytoplasm, elongated, blunt ended nuclei with fine to coarse stippled chromatin and 3-4 prominent nucleoli, round to angular. Kariomegaly and hyperchromatic nuclei are common. The mitotic index is high (7-9 bizarre mitosis per high power field). At trichrome stain, the tumor cell cytoplasm is red. The microscopic findings are compatible with a high grade vascular leiomyosarcoma. The immunohistochemistry is pending to confirm smooth muscle differentiation.

**Poster Number:** D-99

**Section:** Diagnostic Pathology  
**Keyword:** Forensic Veterinary Pathology

**NECROPSIES OF FOUR CATS SEIZED FROM A HOARDING SITUATION**

A.E. Sarfaty¹ and S.P. McDonough¹  
¹Cornell University College of Veterinary Medicine, Ithaca, NY

We describe necropsies of 4 adult cats seized from a large scale hoarding case (685 cats). By describing the cause of death among these cats in a retrospective case study, we hope to aid in future veterinary forensics investigations. 3 of the 4 cats submitted presented in a severely emaciated state (BCS 1/9) due to a combination of primary undernutrition, infectious disease, and impaired immunity. Infective agents causing upper and lower respiratory infections found included Feline Calicivirus, Feline Herpesvirus-1, *Streptococcus zooepidemicus*, and *Neisseria* spp., with *Escherichia coli* as a probable postmortem contaminant. One cat had a history of testing positive for Feline Immunodeficiency Virus, and another for Feline Leukemia Virus (supported by lymphoproliferative changes in the lung and spleen). Several cats had ectoparasites and one had *Aelurostrongylus abstrusus*. There was no evidence of concurrent disease that could cause cachexia to explain the emaciated state of these cats, and their gastrointestinal tracts were empty, indicating they had not eaten for at least 48 hours. A complete absence of omental and visceral fat, along with gelatinous bone marrow, supports the conclusion of primary undernutrition. Primary undernutrition, repeated infection, and impaired immune function have an interrelationship in which each element can affect the other two. Understanding of this interrelationship is important when evaluating animals with histories of neglect. The owner of these cats was convicted of felony animal abuse via a deferred prosecution agreement. Due to compliance with pretrial requirements, the owner was ultimately released from the agreement and all charges were dropped.

**Poster Number:** D-100
AN EFFORT TO INCREASE NECROPSY RATE OF COMPANION ANIMALS IN JAPAN

I. Mitsui
No Boundaries Animal Pathology, LLC, Tokyo, Japan

Necropsy of companion animals is not frequently performed in Japan. The reason for this seems to be multifactorial involving veterinary pathologists, veterinary practitioners, and animal owners. No veterinary school in Japan provides a fee-based necropsy service open to public. This is partly because of paucity of manpower in academia and lack of discussion on developing necropsy service comparable to that in North American veterinary institutions. In addition, most private diagnostic companies in Japan do not have necropsy menu, while they infrequently accept so-called “necropsy in jar” specimens. Japanese veterinary practitioners and animal owners on the other hand are potentially curious about cause of death of animals. Even though Buddhism-based culture seems to have certain unignorable effect on a low necropsy rate, misunderstanding and ignorance about necropsy are likely the most important factors to cause the current situation in Japan.

To advocate the importance and benefit of necropsy of animals, the author established a company providing paid necropsy service and educational seminars in Tokyo in 2012. Eighty nine necropsies so far (as of the end of May 2015) and exchange of information with people of various background gave many hints to increase necropsy rate of companion animals in Japan. Modified cosmetic necropsy, reasonable fee, and clear and thorough communication among pathologists, clinicians, and animal owners are keys to success. However, a long-range strategy should be shared among Japanese pathologists regardless of professional affiliation to reach international level of diagnostic pathology especially in field of companion animal medicine.

Poster Number: D-101

TWO CASES OF NEOPLASIA IN CAPTIVE WHITE SPOTTED BAMBOO SHARKS (*CHILOSCYLLIUM PLAGIOSUM*)

J.F. Rosenberg, M. Haulena, B. Culp, H.M. Evans, G.D. Marty, H.N. Snyman, and S.A. Raverty

Neoplastic disease has infrequently been documented in elasmobranchs, with an approximately 0.4% prevalence. However, two white spotted bamboo sharks (*Chiloscyllium plagiosum*) within the Vancouver Aquarium’s collection were diagnosed with neoplasia on post-mortem evaluation. This report describes a case of rostral squamous cell carcinoma (SCC) and a case of multicentric lymphoma. The first individual was an adult female that had a chronic, non-healing, ulcerative lesion on her rostrum. Ante-mortem diagnostics (blood work, skin scrape, cytology, and aerobic culture) were inconclusive. The shark was ultimately euthanized for quality of life concerns. Histopathology revealed a 10 mm locally invasive...
tumour consistent with SCC. Electron microscopy identified tight junctions and desmosomes that were indicative of an epithelial origin, which confirmed the diagnosis of SCC. The second individual was an adult female of undetermined age. This shark was noted to have an emaciated body condition despite a normal appetite. The individual was removed from the 170,000 L system but succumbed prior to a diagnostic work-up. Gross necropsy revealed a myriad of firm, white, often coalescing nodules that protruded from the surface of the liver. These nodules extended throughout the cut surface and ranged in size from 2 to 15 mm. A single, similar, white nodule was present within the spleen. Histopathology identified an infiltrative, non-encapsulated, densely cellular neoplasm consistent with lymphoma in both liver and spleen. The diagnosis of neoplasia in these individuals emphasizes the importance of a need for diligent recording, reporting, and diagnostics of captive and wild caught elasmobranchs.

**Poster Number**: D-102

**Section**: Diagnostic Pathology  
**Keyword**: Cardiovascular

**VASA VASORUM ARTERIOPATHY IN HYPERTENSIVE CATS**

R. Kohnken, P. Yaxley, and C. Premanandan  
Ohio State University, Columbus, OH

A 14 year-old spayed female mixed longhair cat presented to the Ohio State University Veterinary Medical Center Emergency Care Clinic with a 2 day history of open mouth breathing. There was scant pleural effusion, a grade IV/VI parasternal systolic murmur, and weak femoral pulses. She was placed into an oxygen cage but shortly collapsed. Cardiopulmonary resuscitation (CPR) was unsuccessful. At autopsy, the thorax contained 20 ml transudate and the pericardium contained 20 ml hemorrhage and clotted blood. The proximal main pulmonary artery, ascending aorta, and left atrium were moderately dilated and there was extensive hemorrhage around the great vessels. Pericardial hemorrhage, a focal dorsal tear in the trachea, and associated mediastinal emphysema was interpreted as secondary to CPR trauma. Significant histologic findings included hyperplastic arteriopathy with medial degeneration noted in meningeal vessels and renal interlobular arteries, consistent with systemic hypertension. There was widespread neovascularization and mononuclear inflammation of the vasa vasorum with partial aneurysm of the main pulmonary artery. In animal models of hypertension, oxidative stress on the vasa vasorum results in local hypoxia, which then induces vascular remodeling of the vasa vasorum, main pulmonary artery, and aorta. This lesion and pathogenesis is thought to contribute to atherosclerosis and aortic dissection in people with chronic hypertension. Examination of additional cats indicates that inflammation and neovascularization of the vasa vasorum might be an under-recognized lesion of hypertension in cats. Additionally, we suggest that the main pulmonary arterial rupture in the index case was secondary to vasa vasorum dysfunction.

**Poster Number**: D-103

**Section**: Diagnostic Pathology  
**Keyword**: Infectious Disease
COMPLETE SPLENIC ABSCESSION WITH SEPTIC PERITONITIS IN A HUNTING DOG

K. Schlicher, K. Wycislo, M. Bates, P. Roady, and A. Barger
University of Illinois College of Veterinary Medicine, Department of Pathobiology and the Veterinary Diagnostic Laboratory

A 9 year old female intact German Wirehaired pointer dog presented for progressive weight loss over two months with intermittent vomiting and lethargy. The patient led an active hunting lifestyle and had recently travelled for a competition. Physical examination was within normal limits, and the patient was afebrile. Abdominal ultrasound revealed a large fluid-filled, well-encapsulated hypoechoic mass caudal to the fundus of the stomach, as well as small intestinal thickening and a moderate amount of free abdominal fluid. Fine needle aspiration of the well-encapsulated mass revealed marked septic suppurative inflammation with many coci bacteria. Cytologic evaluation of the free abdominal fluid similarly revealed septic suppurative inflammation with cocci bacteria. The owner elected humane euthanasia. Necropsy revealed the absence of a recognizable spleen with a large fluid-filled fluctuant mass present in the anatomic location of the spleen. Histological examination of the mass was consistent with a large abscess, and a heavy and pure population of Staphylococcus pseudintermedius was cultured. With no history of splenectomy, it is presumed the splenic parenchyma was entirely effaced by an abscess, which resulted in severe septic peritonitis. Splenic abscession in the dog is rare, especially to the extent seen in this patient, but has been reported secondary to penetrating wounds, migrating foreign bodies, or hematogenous spread. Although a direct cause of the abscession was not identified in this patient, it is speculated a penetrating wound or migrating foreign body was the cause given the patient’s active hunting lifestyle.

Education Focused Scientific Poster Presentations

Poster Number: ED-1

Section: Education
Keyword: Bone and Joint

THE IMPORTANCE OF ERGONOMICS IN MINIMIZING POSTURAL STRESS AND ASSOCIATED PATHOLOGY

M. Camus
University of Georgia, Athens, GA

Prolonged microscope use has been associated with chronic pain, primarily in the neck, back, shoulders, and upper extremities for over a century. Several interrelated pathophysiologic mechanisms have been proposed for causing associated symptoms, including repetitive mechanical stress, tissue ischemia, and accumulation of toxic metabolites secondary to inflammation and tissue injury. Prolonged neck flexion may induce laxity and weakness of muscles, tendons, and ligaments, often resulting in a forward leaning posture and dysfunction of neck extensors. The development of microscopes with tilting heads and telescoping eyepieces has potentially lessened problems associated with postural stress. However, the
use of traditional, less expensive, fixed angle microscopes often persists in laboratories and at multiheaded teaching scopes. Additionally, the concurrent use of computers often further compounds positional stress. Microscopists must be proactive in preventing injury through postural awareness and utilization of appropriate workstation design. The CDC recommends no more than 5 hours of microscopy per day and urges microscopists to take frequent short breaks (3 to 5 minutes per every 20 to 30 minutes of microscopy) during which workers stretch and/or perform light exercise. According to the U.S. Bureau of Labor and Statistics, work related musculoskeletal injuries cost American employers over 20 billion dollars in 2010 for worker’s compensation, medical bills, and lost revenue. Both employees and employers must take care to properly prevent, diagnose, and treat musculoskeletal disorders associated with prolonged postural stress.

**Poster Number:** ED-2

**Section:** Education  
**Keyword:** Education

### BUILDING AN INTERNAL PATHOLOGY TECHNICIAN CERTIFICATION PROGRAM AT MPI RESEARCH

C. Hollinger$^{1,2}$, S. Streeter$^4$, P. Ross$^1$, K. Nelson$^{1,2}$, and D. Patrick$^{1,2}$

$^1$MPI Research, Mattawan, MI, USA; $^2$Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA

Attracting and retaining highly skilled technical staff impacts the efficiency and efficacy of pathology departments. In addition to qualifications from national certification programs, on-the-job training increases technician skills, quality, and relevance; however, the value of such experience can be challenging to quantify and risks being overlooked. In an effort to better recognize abilities of technical staff, promote technician advancement, and contribute to departmental and individual improvement, technician leaders and pathologists at MPI Research designed an internal certification program for technicians in necropsy, trimming, and histology sections in 2014. Additional anticipated benefits were to increase technician-pathologist interaction and support exploration of scientific and/or procedural questions through focused projects. The certification requires 1) $\geq 15$ science/mathematics college credits, 2) 2 one-month cross-training in another laboratory section, 3) participation in and presentation of a project, 4) successful completion of a 120-question exam, 5) work experience of 2-6 years in the laboratory (time dependent on prior academic degree), and 6) final approval from the applicant’s supervisor and a pathologist. In addition to individual and departmental development, successful completion yields an increase in the individual’s compensation. Seven technicians (3 necropsy, 3 trimming, 1 histology) successfully completed the program in its first year. All had prior bachelor’s degrees and the average exam score was 89.9%. Six projects focused on necropsy and one on histology; including updates to tissue collection schemes, injection site collection, ocular dissection, and perfusion pump procedures. This certification program succeeds in recognizing and rewarding technical staff, fostering professional growth, and propelling ongoing departmental improvements.

**Poster Number:** ED-3

**Section:** Education  
**Keyword:** General Topic
AUGMENTING GENERAL PATHOLOGY LABS WITH CASE REPORTS
AUTHORED BY SENIOR VETERINARY STUDENTS

A. Fales-Williams, M. Gustafson, J. Hostetter, and J. Danielson
College of Veterinary Medicine, Iowa State University, Ames, Iowa

To augment learning, VM1 students in general pathology at Iowa State University College of Veterinary Medicine were offered case reports (Case Correlation Assignments (CCAs)) authored by senior necropsy students (VM4). CCAs selected for use in the VM1 course provided high educational content and connection to weekly lab topic. Each week, VM1s could choose a CCA and answer a specific question for one point. Questions ranged from stating unfamiliar medical terms, creating a three-step pathogenesis, or recognizing lesions. After the semester, VM1 students were surveyed; 52/123 (42.3%) students responded. 75% of students indicated they read CCAs for at least half of the labs; 56.9% of respondents sometimes read more than one per week. Reading CCAs was helpful (55.8%) or somewhat helpful (40.4%) and interesting (73.1%) or somewhat interesting (26.9%) to most of the class. The CCAs often covered information beyond the scope of VM1 classwork. When asked if these new concepts created negative stress, 90.4% answered “no;” time constraints were the predominant negative response. VM1s frequently commented they didn’t fully understand CCAs but were inspired to look things up. Students were motivated to read CCA’s by earning points (70.6%), curiosity (17.6%) and/or the CCAs’ clinical orientation (11.8%). Interestingly, while students listed many positive attributes for reading CCAs, they could not recall an example of remembering something during an exam they had learned from a CCA. Students indicated that CCAs helped them make connections between what they learned in class and the “real-world,” and particularly helped them learn and retain terminology.

Experimental Disease Focused Scientific Poster Presentations

Poster Number: Poster E-1

Section: Experimental Disease Focused Scientific Session I
Keyword: Ferret

CROSS-DISCIPLINARY SYNERGY: COMBINING HISTOPATHOLOGY AND SYSTEMS BIOLOGY TO CHARACTERIZE EXPERIMENTAL PANDEMIC H1N1 INFLUENZA INFECTIONS IN FERRETS

D. Gasper¹, J. Tisoncik-Go³ J. Kyle⁴, A. Eisfeld¹, C. Selinger³, M. Hatta¹, J. Morrison³, E. Zink⁴, Y. Kim⁴, A. Schepmoes⁴, S. Purvine⁵, K. Weitz⁴, R. Green¹, S. Tilton⁵, B. Webb-Robertson⁶, K. Waters⁴, T. Metz⁴, R. Smith⁴, Y. Kawaoka¹,², M. Suresh⁵, L. Josset², and M. Katze³

¹Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA, ²Laboratoire de Virologie, Centre de Biologie Est des Hospices Civils de Lyon, Université Claude Bernard Lyon 1, Lyon, France, ³Department of Microbiology, University of Washington, Seattle, WA, USA, ⁴Fundamental & Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA, ⁵Environmental Molecular Sciences Laboratory, Pacific
Northwest National Laboratory, Richland, WA, USA, 6National Security Directorate, Pacific Northwest National Laboratory, Richland, WA, USA, 7ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama; Japan Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo; Japan Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo; Japan; Laboratory of Bioresponses Regulation, Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Japan

The domestic ferret (Mustela putorius furo) is an important animal model for influenza pathogenesis and transmission research. Recent advances in the sequencing and annotation of the ferret genome facilitate complex analyses of the molecular networks underlying ferret physiology, and enable mechanistic investigations into disease pathogenesis that were previously impractical. Here we report and compare the results of histologic and quantitative lipidomic, metabolomic, proteomic, and transcriptomic analyses of respiratory tissues from ferrets experimentally infected with pandemic influenza A/Brevig Mission/1/1918 and A/California/04/2009. Histologic examination of trachea and lung at 1, 3, and 8 days after infection with each virus revealed distinct types and patterns of lesions in the trachea, medium-small caliber airways, and alveoli. These patterns were highly associated with virus subtype, alterations in gene expression, and changes in abundance of lipids, metabolites, and proteins during the course of infection. Digital cell quantification utilized the transcriptomic data to infer the molecular phenotype of innate and adaptive immune cell populations observed histologically, and to characterize dynamic population-level changes in inflammatory cells over the course of infection. While histologic assessment provides the basis for disease characterization, systems biology analyses can provide an important molecular context regarding the tissue environment and nature of host responses that cannot be directly observed. The combination of conventional histopathology and systems biology analyses is powerful and synergistic, and allows for a depth and breadth of disease assessment that cannot be achieved independently by each modality.

Poster Number: E-2

Section: Experimental Disease
Keyword: Immune System

INDUCED BRONCHUS-ASSOCIATED LYMPHOID TISSUE AND GERMINAL CENTER FORMATION IN THE MURINE LUNG FOLLOWING INFLUENZA A VIRUS INFECTION

K.N. Gibson-Corley, A.W. Boyden, L. Tygrett, and T.J. Waldschmidt
Department of Pathology, Roy J. & Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA

Induced bronchus-associated lymphoid tissue (iBALT) develops within the lung of both humans and mice following an adaptive immune response upon exposure to certain classes of antigens. iBALT has been documented to develop with Influenza A virus (IAV) infection but the kinetics of this localized immune response have yet to be documented. Using both flow cytometry and immunohistochemistry we have identified the structure, composition and development of B cells and iBALT over time in mice intranasally infected with an H1N1 IAV. Early in infection (days 6-12) B cells accumulate within the lung,
primarily around blood vessels, and are very loosely organized. By flow these are mature B cells and there is no evidence of germinal center cells. As time progresses to days 18 and 24, B cells more tightly organize around medium to large airways and have a well-defined lymphoid structure complete with Bcl-6 positive, PNA\textsuperscript{hi}, CD95\textsuperscript{hi}, IgM\textsuperscript{hi} germinal center B cells which persist up to and likely beyond day 50 of infection. These findings are of interest as Influenza virus is constantly undergoing antigenic changes which can result in epidemics and pandemics. Understanding the role of B cells in iBALT can help us identify novel targets for vaccine development, as it is a localized tertiary lymphoid organ that can be considered a local site for immune memory.

**Poster Number:** E-3

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**THE ROLE OF CLUB CELL SECRETORY PROTEIN (CC10) IN RESPIRATORY SYNCYTIAL VIRUS INFECTION**

S.S. Alnajjar\textsuperscript{1}, A. Larios-Mora\textsuperscript{1}, A. van Geelen\textsuperscript{1}, J.M. Gallup\textsuperscript{1}, A. Pilon\textsuperscript{2}, and M.R. Ackermann\textsuperscript{1}  
\textsuperscript{1}Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa USA, \textsuperscript{2}Therabron Therapeutics, Inc., Rockville, MD USA

Club cells are one of the predominant cell types in the bronchial tree. Club cell secretory protein (CC10), is an immunomodulator protein that can alter viral infection. However, its exact role in the lung has not been fully investigated. In this study, we determine the levels of CC10 in lung epithelia of lambs with and without Respiratory Syncytial Virus (RSV) infection in order to better understand the extent of expression of CC10.

Neonatal lambs were nebulized with RSV M37 (6 ml of RSV M37 at 1.3 x 10\textsuperscript{7} FFU/ml). The lambs were euthanized on days 1, 3, 4, 6, and 8 after viral infection. CC10 was detected by immunohistochemistry on formalin-fixed paraffin-embedded (FFPE) lung tissue sections and CC10 mRNA levels were measured by RT-qPCR in RNA extracted from frozen lung samples.

With RSV infection, there was a significant reduction in CC10 mRNA expression compared to the control uninfected group. CC10 antigen in control, non-infected lambs was present in the apical cytoplasm of most epithelial cells lining bronchioles and in occasional cells lining bronchi. CC10 antigen was reduced in RSV-infected lung tissues compared to control. Furthermore, bronchioles within RSV lesions completely lacked CC10 immuno-reactivity.

These results indicate that CC10 decreases with increased severity of RSV infection and associated lesions, indicating a possibly significant role of CC10 in modulating lung infection.

**Poster Number:** E-4

**Section:** Experimental Disease  
**Keyword:** Infectious Disease
JNJ-49214698 EFFICACY IN A NEONATAL LAMB MODEL OF RSV INFECTION

S.S. Alnajjar1, A. Larios-Mora1, A. Geelen1, J.M. Gallup1, D. Roymans2, A. Kouil2, P. Rigaux2, and M.R. Ackermann1

1Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, Iowa USA, 2Janssen Pharmaceuticals, Inc., Beerse, Belgium

Respiratory syncytial virus (RSV) remains the leading viral agent of acute respiratory tract infection, subsequent hospitalization, and death in young children. This study determines the efficacy of RSV fusion-inhibitor, JNJ-49214698, in a neonatal lamb model of RSV that mimics RSV disease in infants.

Lambs were randomly assigned to five different groups. Three groups were all treated daily with JNJ-49214698 at 25 mg/kg by intragastric gavage. The first group was treated prophylactically, starting 1 day before RSV challenge. The second and the third groups were started on treatment one day and three days after viral infection, respectively. The other two groups served as positive and negative RSV controls. All groups were nebulized with RSV M37 (6 ml of RSV at 1.3 X 10⁷ FFU/ml), except the control negative group that was nebulized with mock media. All lambs were euthanized six days post-infection.

Assessed parameters included: Lung gross and microscopic lesions, viral antigen (by immunohistochemistry), viral titers and mRNA levels in lung and bronchoalveolar lavage fluid (BALF). The prophylactically-treated group lacked RSV lesions and titers. Lambs receiving daily JNJ-49214698 beginning at days one and three after RSV infection had significantly reduced lesions and viral parameters in comparison to the control, RSV-infected group. The decrease in viral infection was correlated with the time of onset of treatment; the earlier the onset of daily treatment, the less the viral infection.

In conclusion, JNJ-49214698 has a protective effect against RSV infection and decreases viral infection and pathology when used during established infection.

Poster Number: E-5

Section: Experimental Disease
Keyword: Respiratory System

CELLULAR DISTRIBUTION OF DIPEPTIDYL PEPTIDASE-4 (CD26) IN THE RESPIRATORY TRACT: IMPLICATIONS FOR ANIMAL MODELS OF MIDDLE EAST RESPIRATORY SYNDROME

D.K. Meyerholz1, A.M. Lambertz1, P.B. McCray, Jr.2

1Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA, 2Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA

Dipeptidyl peptidase-4 (CD26) is a widely expressed type II transmembrane ectopeptidase that serves as the receptor for Middle East Respiratory Syndrome Coronavirus (MERS-CoV). MERS-CoV has a mortality
rate of nearly 40% and most fatalities occur in patients (>60 years) with co-morbidities such as diabetes, immunodeficiency or lung disease. Lack of autopsy data has hindered understanding of MERS pathogenesis. Murine CD26 does not allow for MERS-CoV replication and models that express human CD26 (hCD26) under ubiquitous (CAAGS) or tissue specific (cytokeratin 18) promoters have been utilized. We studied (IRB approved) CD26 expression in archival human respiratory tract with and without chronic lung disease co-morbidities (e.g. COPD or Cystic Fibrosis) and evaluated this in relation to expected clinical disease in humans and mice. Surface epithelium (nasal cavity and lung airways) had uncommon scattered cellular expression. In contrast, serous cells of submucosal glands had robust staining in the apical cytoplasm and duct lumens that suggested CD26 secretion. In the lung, alveolar macrophages along with type I and II cells had common staining as did subsets of scattered lymphocytes, endothelium of lymphatics and mesothelial cells lining the pleural surface. Chronic lung diseases and tissue remodeling increased CD26 staining in several cell types. The preferential localization of CD26 in the distal lung could be consistent with the radiologic localization of ground glass appearances suggestive of diffuse alveolar damage. Studies in mouse models of MER-CoV may want to compare transgene promoter expression in mouse lung to evaluate the potential similarity of MERS lung disease to humans.

**Poster Number:** E-6

**Section:** Experimental Disease

**Keyword:** Avian

**VELOGENIC AND MESOGENIC NEWCASTLE DISEASE VIRUS PROTEIN WITHIN NEURONS, ASTROCYTES AND MICROGLIA**

S.L. Butt\(^1\), C.L. Afonso\(^2\), V.M.B.D. Moura\(^3\), L. Susta\(^4\), S. Cardenas-Garcia\(^2\), P.J. Miller\(^2\), C.C. Brown\(^1\), and J.B. Stanton\(^1\)

\(^1\)Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA,
\(^2\)Southeast Poultry Research Laboratory, Agricultural Research Service, USDA, Athens, GA, USA,
\(^3\)Animal Pathology, School of Veterinary Medicine and Animal Science, Federal University of Goiás, Goiânia, GO, Brazil,
\(^4\)Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Newcastle disease (ND) is caused by virulent strains of Newcastle disease virus (NDV). NDV strains are classified into pathotypes, from least to most virulent, as lentogenic, mesogenic and velogenic. Velogenic NDV strains are further subdivided into velogenic viscerotropic (VVNDV) and velogenic neurotropic (VNNDV). NDV replication outside of the central nervous system is most often documented in various epithelial cells, macrophages, and lymphocytes; however, the cellular tropism within the brain is less understood. Previous studies, using cell morphology alone, concluded that NDV replication occurs primarily in neurons, but this has not been confirmed by immunophenotyping of the NDV-positive cells and the role of glial cells has not been studied. In this study, double immunohistochemistry was performed to determine the cellular tropism of three NDV strains in the brains of day-old chickens inoculated in the subdural space. Applied strains were a VVNDV (South Africa/08100426/2008 [SA60]), a VNNDV (US/GB/48 [TxGB]), and a mesogenic NDV (US/TX4156/2005 [TX450]). NDV nucleoprotein from all three pathotypes was primarily found in NeuN-positive neurons and GFAP-positive astrocytes, but rarely in RCA-I-positive microglia. These observations suggest that NDV can replicate in cells other than
neurons, including astrocytes and microglia, which could play an important role in neuropathogenesis of different NDV pathotypes.

**Poster Number:** E-7

**Section:** Experimental Disease

**Keyword:** Infectious Disease

**THYROID HYPERTROPHY IN CATS INFECTED WITH HIGHLY PATHOGENIC FELINE IMMUNODEFICIENCY VIRUS**

S.R. Roff1, A.M. Aranyos1, R. Pu1, J.L. Owen2, W.M. Zoll1, and J.K. Yamamoto1
1Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL, 2Department of Physiologic Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL

Twelve cats in a prototype FIV vaccine trial using multi-antigenic peptides (MAP) were challenged with a highly pathogenic subtype B strain of FIV (FIVFC1). Fifteen weeks post challenge, 4 of 11 cats underwent scheduled necropsy and revealed marked lymphoid hyperplasia of the spleen with lymphadenomegaly in both peripheral (axillary) and central (mesenteric) lymph nodes in all (4 of 4) cats with marked bilateral thyroid hypertrophy (up to 6x normal size) in 3 out of 4 cats. Thyroid hypertrophy was transient and not identified in cats necropsied 20-40 weeks post challenge. Twelve to 15 weeks post challenge, 11 of 12 cats that tested positive for FIV (proviral PCR/virus isolation) developed elevations in either free T4 (8 of 11) or TSH (2 of 11) without elevations in total T4. Elevations in free T4 and TSH correlated to inversion of the CD4/CD8 T-cell ratio and were represented in all groups. One cat (SBA) was protected from challenge (negative by proviral PCR/virus isolation) and did not demonstrate either thyroid hypertrophy or elevations in free T4 or TSH. These results demonstrate a novel, transient thyroid hypertrophy in cats infected with highly pathogenic FIVFC1. Findings suggest transient thyroid hypertrophy via immune dysregulation stimulating TSH release by the pituitary. Although this finding is considered incidental to the current vaccine studies, it may warrant further investigation to determine whether recrudescence of highly pathogenic strains of FIV may result in immune dysregulation potentially leading to thyroid hypertrophy, dysplasia or autoimmune thyroid disease in aged cats chronically infected with FIV.

**Poster Number:** E-9

**Section:** Experimental Disease

**Keyword:** Infectious Disease

**SUSCEPTIBILITY OF DEER MOUSE (PEROMYSCUS MANICULATUS) TO VESICULAR STOMATITIS NEW JERSEY VIRUS INFECTION VIA BLACK FLY (SIMULIUM VITTATUM) BITE**

University of Georgia, Athens, GA
The natural transmission of *Vesicular stomatitis New Jersey virus* (VSNJV), an arthropod-borne virus, is not completely understood. Rodents may have a role as reservoir or amplifying hosts and juvenile deer mice (*Peromyscus maniculatus*), a native New World rodent, can develop viremia following intranasal or intradermal inoculation. In the present study, juvenile and nesting deer mice were exposed to VSNJV-infected black fly (*Simulium vittatum*) bites. Naive black flies were allowed to feed on nesting mice 1 and 2 days following initial infection. Juvenile mice developed severe neurological signs by 6 to 8 days post-inoculation (DPI) and central nervous system lesions (CNS) were located mainly in spinal cord and brain stem. These were characterized by leptomeningitis, encephalitis and neuronal necrosis. VSNJV-antigen distribution in juveniles indicated that viral retrograde axonal transport was the most likely route into the CNS. In contrast, a hematogenous route was also involved in nesting mice, as all of these mice developed viremia and had a widespread antigen distribution in CNS and other tissues on 2 DPI. VSNJV was also recovered from naive flies fed on viremic nesting mice. This is the first report of viremia in a natural host following infection with VSNJV via insect bite and of an insect becoming infected with VSNJV by feeding on a viremic host. These results, along with histopathology and immunohistochemistry, show that nesting mice are susceptible to widespread dissemination of VSNJV following VSNJV-infected black fly bite and are a potential VSNJV reservoir or amplifying host.

**Poster Number:** E-10

**Section:** Experimental Disease  
**Keyword:** Avian

**AN EXPERIMENTAL MODEL FOR STUDYING THE PATHOGENESIS OF A NEWLY EMERGENT TURKEY ARTHRITIS/TENOSYNOVITIS REOVIRUS IN TURKEYS**

T.A. Sharafeldin, S.K. Mor, S.M. Goyal, and R.E. Porter  
Veterinary Population Medicine department, University of Minnesota, Saint Paul, Minnesota

Recently, in 2011, turkey reoviruses were isolated from tendons and synovial fluids of >15-week-old lame male turkeys with swollen joints and occasionally ruptured leg tendons in Midwest, USA. These newly isolated reoviruses were tentatively called turkey arthritis/tenosynovitis reoviruses (TARV) and previously isolated reoviruses from intestinal contents and feces of turkeys with enteric syndromes were called turkey enteric reoviruses (TERV). TARV were genetically distinct from chicken arthritis reoviruses (CARV). The present experimental model enabled studying the pathogenesis of TARV and cytokine immune responses in turkeys. TARV showed unique capability to induce significantly higher tenosynovitis histologic inflammation scores compared with TERV and CARV which induced minimal scores. Clinical lameness was first displayed at 8 weeks of age in TARV-inoculated turkeys at 1 week old via oral route. TARV displayed the greatest replication in intestines and bursa of Fabricius then in leg tendons of turkeys. Viral infection mediated effective antiviral cytokines immune responses that remarkably limited the viral replication in intestines. Furthermore, the viral infection mediated a dominant significantly elevated T helper-1(Th1) cytokine response in intestines and tendons and minimal Th2 and Th17 cytokine response during the early stage (2 weeks) of infection. This newly developed reproducible experimental model provided early end points that are indicative for the disease pathogenicity, associated the development of a new grading system for histologic tenosynovitis.
which can be used in wide variety of experimental models, proved the significant unique pathogenicity of the newly isolated TARV in turkeys and added significant knowledge to TARV pathogenesis and immune response.

**Poster Number:** E-11

**Section:** Experimental Disease  
**Keyword:** Respiratory System

**PATHOGENESIS OF PASTEURIELLA MULTOCIDA LIPOPOLYSACCHARIDE IN THE RESPIRATORY TRACT OF RABBITS**

Veterinary Pathobiology Group, Universidad Nacional de Colombia, Bogotá D.C., Colombia

*P. multocida* is a gram negative coco-bacillus considered part of the normal flora of the upper airways and causes disease in a wide range of animals. The pathogenic mechanisms by which this bacterium causes disease are not completely elucidated. This work describes the importance of *P. multocida* lipopolysaccharide (LPS) during the course of the disease in rabbits, how the LPS interacts with cells of the respiratory tract, and possible mechanisms responsible for the pulmonary lesions it causes. Rabbits were instilled intra-nasally with LPS of *P. multocida*. Nasal cavity and lung samples were processed for light microscopy, lectin-histochemistry (LHC) and transmission electronmicroscopy (TEM). Increase of goblet cells (GC) activation and infiltration of polymorphonuclear (PMN) cells within the respiratory epithelium were the most relevant changes in nasal cavity; a predominantly interstitial pattern was the main lesion found in the lungs. By LHC and/or TEM, LPS was observed admixed with mucus, and attached to cilia, microvilli and cell membrane of respiratory cells. Moreover, it was seen internalized within the cytoplasm of GC, ciliated and glandular cells in the nasal septum, and within the cytoplasm of bronchiolar epithelial and alveolar epithelial cells in the lung. In addition, a positive staining was observed inside PMNs and surrounding the membrane of erythrocytes of the samples studied. These results indicate that the LPS of *P. multocida* adheres to and invades the nasal respiratory epithelium, reaches blood circulation, and primes PMNs that travel to the lung where it causes diffuse alveolar damage that leads to interstitial pneumonia in rabbits.

**Poster Number:** E-12

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**GLYCOINHIBITORS OF ADHERENCE OF STREPTOCOCCUS AGALACTIAE TO INTESTINAL EPITHELIUM IN TILAPIA (ORECHROMIS SP.)**

P. Barato, G.M. Vasquez, and C. Iregui  
Group of Veterinary Pathobiology, Universidad Nacional de Colombia, Bogotá, Colombia
Streptococcosis is one of the most important diseases in tilapia (*Oreochromis* sp.) industry on the world; it causes serious economic losses in fish aquaculture. In tilapia, the disease is induced by *Streptococcus agalactiae*, *S. iniae*, *S. dysgalactiae* and *Lactococcus garvieae*. Vaccines against *S. agalactiae* have a limited value (homologous biotype protection), and *S. iniae* and *S. agalactiae* have developed resistance against some antibiotics. As alternative and complementary strategy, glycobiological anti-adhesive therapy to inhibit the adhesion of *S. agalactiae* to host has been proposed to prevent the disease. On the other hand, fish species like cachama (*Piaractus brachypomus*) are resistant to streptococcosis in spite of living with and eating diseased tilapia. In this study, by lectin histochemistry, the carbohydrate patterns on the apical surface of the intestinal epithelium of healthy tilapias, tilapias with streptococcosis, intestinal explants of tilapia infected with *S. agalactiae* and healthy cachamas were described for the first time. From the glycobiological point of view, the presence of Gal/GalNAc/Lac/LacNAc in the intestine of tilapias and their absence in cachamas could be related with tilapia susceptibility or cachama resistance to be infected by *S. agalactiae*. Also, in an *ex vivo* infection model in tilapia intestine, we inhibited the adherence of *S. agalactiae* using three sugars and one lectin selected from the lectin histochemistry study. These molecules are the basis for the manufacture of anti-adhesive medicaments for prevention, treatment or control against streptococcosis in fish.

**Poster Number:** E-13

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**EFFECT OF *AMBLYOMMA MACULATUM* FEEDING ON THE CUTANEOUS IMMUNE RESPONSE TO *RICKETTSIA PARKERI* INFECTION IN A MURINE MODEL**

K.H. Banajee and K.R. Macaluso  
Department of Pathobiological Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA

*Rickettsia parkeri* is an emerging eschar-causing human pathogen and spotted fever group (SFG) rickettsial species transmitted by the Gulf coast tick, *Amblyomma maculatum*. Tick saliva contains many pharmacologically active molecules that have been shown to alter both the cellular and humoral components of the innate and adaptive immune systems in murine models. However, the effect of tick feeding on the mammalian cellular and humoral immune response at the cutaneous interface has not been examined for *R. parkeri* rickettsiosis. Our hypothesis is that *A. maculatum* feeding modifies the mammalian host response to *R. parkeri* thereby enhancing pathogenesis. In order to test this hypothesis, mice were intradermally inoculated with *R. parkeri* both in the presence and absence of nymphal *A. maculatum* feeding, and sacrificed 6, 24, and 48 hours post-inoculation. The skin at the inoculation site and local lymph nodes were collected in order to assess inflammatory cell infiltrates and cytokines at the inoculation site, and rickettsial dissemination. Preliminary results indicate that six hours after rickettsial inoculation there is marked neutrophilic inflammation with macrophages infiltrating the inoculation site by 24 hours and resolution of inflammation by two days post inoculation. Tick feeding during inoculation resulted in upregulation of Th2 cytokines and downregulation of Th1 response to *R. parkeri*, increased rickettsial transmission and dissemination compared to needle inoculation alone.
These results confirm that tick feeding enhances the pathogenicity of SFG *Rickettsia* and indicate that strategies aimed at blocking tick immunomodulatory factors could be beneficial in disease prevention.

**Poster Number:** E-14

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**FATAL *PLASMODIUM KNOWLESI* INFECTION IN THREE BOLIVIAN SQUIRREL MONKEYS**

Centers for Disease Control and Prevention, Atlanta, GA

Human malaria is the most devastating parasitic disease worldwide, with an estimated 198 million cases and 584,000 deaths in 2013. Classically, four host adapted parasites, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*, are the causes of human malaria. In the 1930’s an Old World monkey adapted *Plasmodium* species, *P. knowlesi*, was recognized to be permissive to human infection in research and therapeutic settings. Since the 1960s sporadic natural infections of *P. knowlesi*, the fifth human malaria, have been reported as rare zoonotic events restricted to Southeast Asia. In 2004 a large focus of naturally acquired *P. knowlesi* infections was identified, and since then, increased specificity of detection methods has allowed for recognition of this parasite as a significant human pathogen that can cause severe malarial disease. Very little is known about the pathophysiology of severe *P. knowlesi* malaria in people, and infection is possible in most primate species allowing for research on pathogenesis and immunologic response, and characterization of vaccine and therapeutic efficacy. Three adult Bolivian squirrel monkeys (*Saimiri boliviensis boliviensis*) were inoculated with frozen blood containing *P. knowlesi* and subsequently treated with oral chloroquine. All animals developed severe parasitemia and, due to treatment failure, succumbed to infection 5–40 days after inoculation. Gross, histologic and immunohistochemical evaluation revealed a widely disseminated infection with innumerable parasitized red blood cells (pRBC), and abundant hemozoin pigment. Furthermore, abundant pRBCs were present within capillaries of the brain, similar to what has been noted in a fatal human case. Herein the gross, histologic, immunohistochemical and electron microscopic findings of fatal malaria in squirrel monkeys are described and compared to findings in human malaria.

**Poster Number:** E-15

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**HISTOMORPHOLOGICAL ASPECTS OF EXPERIMENTAL *TRYPANOSOMA BRUCEI* INFECTION**

T. Carvalho, F. Rijo-Ferreira, S. Trindade, and L.M. Figueiredo  
Instituto de Medicina Molecular, Faculdade de Medicina, Lisbon, Portugal
African Trypanosomiasis, sleeping sickness, is a neglected tropical disease affecting humans and animals. While parasite distribution in brain has been extensively studied, less is known about other organs. Herein, the goal was to assess *Trypanosoma brucei* distribution in different organs of the mouse, for which C57BL/6J were infected, sacrificed after 8 and 21 days, and their organs processed for histological analysis, immunohistochemistry (with anti-VSG polyclonal antibodies), transmission electron microscopy and molecular biology. Besides the choroid plexuses, *T. brucei* was present in the stromal compartment of heart, lung, but mainly in adipose tissue, pancreas, and epididymis. In the later, stroma was expanded due to infiltration by parasites and mononuclear inflammatory cells, with intense staining for VSG, and parasite DNA levels were high, suggestive of high parasite load. However, RNA parasite levels were low and electron microscopy showed rare intact parasites in the epididymis, displaying abnormal morphology and with numerous loose flagella (extracellular). This contrasted with the pancreas that showed increasingly higher parasite load, with preservation of cell integrity. In conclusion, we highlight the value of pathology in the characterization of mouse models of human disease, further showing that a combination of different histological methods may reveal additional and rather interesting phenotypes, otherwise undisclosed. Trypanosomes homing to the epididymis fail to thrive and die; and this organ is therefore an unlikely source of disease relapse. Pancreas, on the other hand, showed increasingly higher parasite load and pancreatic failure might be the proximal cause of death in the mouse model of African trypanosomiasis.

**Poster Number:** E-16

**Section:** Experimental Disease

**Keyword:** Infectious Disease

### MATRIX METALLOPROTEINASE 2 ACTIVITY IN SCRAPIE PERMISSIVE AND NONPERMISSIVE CULTURED SHEEP MICROGLIA

J.B. Stanton¹, V.R. McElliott¹, J.F. Muñoz-Gutiérrez², and D.A. Schneider²,³

¹Department of Pathology, University of Georgia, Athens, GA, ²Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, ³United States Department of Agriculture, Agricultural Research Service, Animal Disease Research Unit, Pullman, WA

Prion diseases, including scrapie, bovine spongiform encephalopathy, and chronic wasting disease, are invariably fatal neurodegenerative diseases. Prions are primarily, if not solely composed of abnormally folded cellular prion proteins; thus, expression of normal cellular prion protein is required for prion permissiveness. However, evidence indicates that the expression of cellular prion protein is insufficient for permissibility and that there are additional determinants of permissibility. To identify genes and pathways associated with prion permissibility, we previously compared the transcriptomes of two clones of an ovine microglial cell line: one permissive and the other nonpermissive to scrapie prion infection. The clones were derived from the same parental cell line; thus, they are genetically identical, including the prion gene sequence. The results suggested that several extracellular processes, including proteolysis, were significantly differentially regulated, with a potential for increased matrix metalloprotease 2 (MMP2) activity in scrapie-nonpermissive cells. To verify the transcriptomic data, extracellular MMP2 activity in the two clonal cell lines was measured by zymography and determined to be decreased in the scrapie permissive clone. To expand these results, MMP2 activity was measured in additional clones and sublines of ovine microglia, and MMP2 activity was lowest in the most scrapie-
permisive cells. These results provide additional support that MMP activity and extracellular matrix remodeling may significantly influence prion permissibility. Functional studies are underway to test these associations for causality.

**Poster Number:** E-17

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**SCRAPIE TRANSMITS TO WHITE-TAILED DEER BY THE ORAL ROUTE AND HAS A MOLECULAR PROFILE SIMILAR TO CHRONIC WASTING DISEASE**

J.J. Greenlee¹, S.J. Moore¹, J.D. Smith¹, R.A. Kunkle¹, and M.H. West Greenlee²  
¹Virus and Prion Research Unit, National Animal Disease Center, ARS, USDA, Ames, IA, ²Iowa State University, Ames, Iowa

The purpose of this work was to determine susceptibility of white-tailed deer (WTD) to the agent of sheep scrapie and to compare the resultant PrPSc to that of the original inoculum and chronic wasting disease (CWD). We inoculated WTD by a natural route of exposure (concurrent oral and intranasal (IN); n=5) with a US scrapie isolate. All scrapie-inoculated deer had evidence of PrPSc accumulation. PrPSc was detected in lymphoid tissues at preclinical time points, and deer necropsied after 28 months post-inoculation had clinical signs, spongiform encephalopathy, and widespread distribution of PrPSc in neural and lymphoid tissues. Western blotting (WB) revealed PrPSc with 2 distinct molecular profiles. WB on cerebral cortex had a profile similar to the original scrapie inoculum, whereas WB of brainstem, cerebellum, or lymph nodes revealed PrPSc with a higher profile resembling CWD. Homogenates with the 2 distinct profiles from WTD with clinical scrapie were further passaged to mice expressing cervid prion protein and intranasally to sheep and WTD. In cervidized mice, the two inocula have distinct incubation times. Sheep inoculated intranasally with WTD derived scrapie developed disease, but only after inoculation with the inoculum that had a scrapie-like profile. The WTD study is ongoing, but deer in both inoculation groups are positive for PrPSc by rectal mucosal biopsy. In summary, this work demonstrates that WTD are susceptible to the agent of scrapie, two distinct molecular profiles of PrPSc are present in the tissues of affected deer, and inoculum of either profile readily passes to deer.

**Poster Number:** E-18

**Section:** Experimental Disease  
**Keyword:** Nervous System

**TRANSMISSION OF CHRONIC WASTING DISEASE TO SENTINEL REINDEER (RANGIFER TARANDUS TARANDUS)**

USDA, ARS, National Animal Disease Center, Ames, IA
Chronic wasting disease (CWD) is a naturally-occurring, fatal neurodegenerative disease of North American cervids. Reindeer (Rangifer tarandus tarandus) are susceptible to CWD following oral challenge, but CWD has not been reported in free-ranging caribou (Rangifer tarandus caribou) or farmed reindeer. Potential contact between CWD-affected cervids and Rangifer species that are free-ranging or co-housed on farms presents a potential risk of CWD transmission. The aims of this study were to 1) investigate the transmission of CWD from white-tailed deer (Odocoileus virginianus; CWDwtd), mule deer (Odocoileus hemionus; CWDmd), or elk (Cervus elaphus nelsoni; CWDelk) to reindeer via the intracranial route, and 2) to assess for direct and indirect horizontal transmission to non-inoculated sentinels. Three groups of 5 reindeer fawns were challenged intracranially with CWDwtd, CWDmd, or CWDelk. Two years after challenge of inoculated reindeer, non-inoculated control reindeer were introduced into the same pen as the CWDwtd inoculated reindeer (n=4) or into a pen adjacent to the CWDmd inoculated reindeer (n=2). Reindeer were allowed to develop clinical disease. At death/euthanasia a complete necropsy examination was performed, including immunohistochemical testing of tissues for disease-associated CWD prion protein (PrP\textsuperscript{CWD}). Intracranially challenged reindeer developed clinical disease from 21 months post-inoculation (MPI). PrP\textsuperscript{CWD} was detected in 5/6 sentinel reindeer although only 2/6 developed clinical disease during the study period (< 57 MPI). We have shown that reindeer are susceptible to CWD from various cervid sources and can transmit CWD to naïve reindeer both directly and indirectly.

**Poster Number:** E-19

**Section:** Experimental Disease  
**Keyword:** Infectious Disease

**BRAIN DERIVED LIPIDS INHIBIT PRION AMYLOID FORMATION IN VITRO**

C.E. Hoover, D.M. Henderson, M. Zabel, and E.A. Hoover  
Prion Research Center, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

The normal cellular prion protein (PrP\textsuperscript{C}) resides in cellular outer membrane lipid rafts and conversion from PrP\textsuperscript{C} to the pathogenic misfolded form is believed to occur at the lipid membrane. Once misfolding occurs, monomers of the pathogenic isoform can assemble into highly stable amyloid fibrils. *In vitro* assays have demonstrated the intimate association between prion conversion and lipids, specifically phosphatidylethanolamine, which is a critical cofactor in the formation of synthetic infectious prions. In the current study, we demonstrate an opposing property of brain lipids, the ability to inhibit amyloid formation *in vitro*. The amyloid seeding assay, real-time quaking-induced conversion assay (RT-QuIC), was used to investigate brain lipid effects on prion amyloid formation. An alcohol based extraction technique was used to remove the lipid content from terminal chronic wasting disease (CWD)-infected white tailed deer brain homogenates. Eliminating lipids increased the sensitivity of RT-QuIC detection of CWD in brain samples by one hundred-fold. Conversely, addition of brain-derived lipid extracts to CWD prion samples inhibited amyloid formation in a dose-dependent manner. Brain-derived lipids also inhibited prion amyloid formation in RT-QuIC reaction seeds derived from lymphoid tissues. Subsequent lipid analysis demonstrated the inhibitory property was restricted to brain polar lipids. This is the first demonstration that a fraction of brain derived lipids directly inhibit prion amyloid formation *in vitro* and highlights the diverse roles lipids play in the prion conversion process. Further
experiments aim to identify the lipid classes or individual lipid species responsible for this inhibitory activity.

**Poster Number:** E-20

**Section:** Experimental Disease  
**Keyword:** Immune System

**EXPLORING ANTI-TUMOR PROPERTIES OF HUMAN CD8+BTLA+ TUMOR INFILTRATING LYMPHOCYTE SUBSET USING NSG MOUSE MODEL**

K. Ritthipichai\(^1,2\), C. Haymaker\(^1\), R. Nurieva\(^2,3\), P. Hwu\(^1\), and C. Bernatchez\(^1\)  
\(^1\)Department of Melanoma Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, TX, \(^2\)Graduate Program in Immunology, University of Texas Graduate School of Biomedical Sciences, Houston, TX, \(^3\)Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX

Clinical trials of human adoptive T-cell therapy (ACT) using autologous ex vivo expanded tumor-infiltrating lymphocytes (TIL) have demonstrated a great potential immunotherapy in stage IV metastatic melanoma. Our recent finding showed a strong association between CD8\(^+\)TIL expressing B-and T lymphocyte attenuator (BTLA) in the infusion product with positive clinical outcome. BTLA is known as an inhibitory molecule expressed by different immune cells. However, emerging evidence shows that BTLA is highly enriched in less-differentiated cells and serves as a T cell differentiation marker. We found that CD8\(^+\)BTLA\(^+\)TIL had superior proliferative capacity and produce more IL-2 following T cell receptor triggering. When ligated with its ligand, herpes virus entry mediator (HVEM), BTLA provided a pro-survival signal with enhancement of AKT phosphorylation. To further explore potential in vivo anti-tumor effects, we used an immunodeficient mouse, NOD scid gamma (NSG), to engraft human derived melanoma and TIL. We found that CD8\(^+\)BTLA\(^+\) exhibited a better tumor burden control and persisted much longer in peripheral blood following TIL infusion as compared with their CD8\(^+\)BTLA\(^-\) counterparts. This suggests that BTLA is not only a T cell differentiation marker, but also confers anti-tumor properties. Therefore, the strategy to preserve BTLA expression may help generate less-differentiated TIL that exhibit enhanced persistence following infusion and resulting in an improved clinical response to ACT.

**§ Poster Number:** E-21

**Section:** Experimental Disease  
**Keyword:** Mammary

**SUPPRESSION OF BREAST CANCER STEM CELLS IN THE MMTV-HER2/NEU MOUSE MODEL BY THE HDAC INHIBITOR AR-42**

L.E. Himmel\(^1,2\), M-W Chao\(^2\), E-C Hsu\(^2\), L.D. Yee\(^3\), S.K. Kulp\(^2\), and C-S Chen\(^2\)  
\(^1\)The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, \(^2\)The Ohio State University College of Pharmacy, Division of Medicinal Chemistry and Pharmacognosy, \(^3\)Wexner Medical Center, Division of Surgical Oncology, Columbus, OH
In many types of cancer, a self-renewing subset of the tumor population described as cancer stem cells (CSC) is linked to tumor recurrence, drug resistance, and metastasis. Thus far, there is a paucity of data validating a mouse model for the investigation of breast cancer stem cells (BCSC) in chemotherapeutic and chemopreventive studies. Mounting evidence suggests that a BCSC subpopulation exists in the MMTV-Her2/neu mouse. The MMTV-Her2/neu mouse model of human breast cancer is superior to xenograft or syngeneic models in that it is immunocompetent and that tumors arise in a stochastic manner. Our experiments in human triple-negative breast cancer cell lines have elucidated a mechanistic link between Notch1 and HDAC8 that may be important in regulating BCSC. We have utilized NT5 mammary tumor cells derived from the MMTV-Her2/neu mouse to predict the in vivo effects of HDAC inhibition on BCSC in this model. In vitro experiments using pan-HDAC inhibitors AR-42 and vorinostat and the HDAC8-selective inhibitor PCI-34051 demonstrated downregulation of stem cell biomarkers and decreased tumorsphere formation. NT5-allografted mice treated with dietary AR-42 showed marked tumor suppression. We have also performed ALDH1 immunohistochemistry to demonstrate putative BCSC populations in mouse Her2/neu tumor sections. Our studies elucidate a unique effect of AR-42 in its capacity to eliminate CSC by modulating the Notch1 signaling pathway. Taken together, our data support the hypothesis that histone deacetylases are involved in regulating the BCSC population and have translational potential to give rise to new therapeutic approaches for breast cancer chemotherapy.

§ Poster Number: E-22

Section: Experimental Disease
Keyword: Liver and Pancreas

THE HISTONE DEACETYLASE INHIBITOR AR-42 SUPPRESSES TUMOR GROWTH IN MOUSE MODELS OF PANCREATIC CANCER

S.E. Henderson1, P.H. Huang2, L.Y. Ding3, T. Bekaii-Saab3, S.K. Kulp4, and C.S. Chen4,5
1Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, Columbus OH, 2Department of Biochemistry and Molecular Biology, National Cheng Kung University, Tainan City, Taiwan (R.O.C.), 3Department of Internal Medicine, College of Medicine, Ohio State University, Columbus OH, 4Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Ohio State University, Columbus, OH, 5Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan (R.O.C.)

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States with a 5-year survival of less than 6% for all stages. This study evaluated the efficacy of AR-42, a novel histone deacetylase (HDAC) inhibitor developed in our laboratory and currently in clinical trials, in suppressing tumor growth in PDAC. AR-42 demonstrated potent anti-proliferative effects on the growth of six human pancreatic cell lines (AsPC-1, COLO-357, Panc-1, MiaPaCa-2, BxPC-3, and SW1990) as measured by MTT assay. Growth suppression in AR-42 treated cells was associated with dose-dependent modulation of proliferation and apoptotic markers, including down-regulation of the apoptotic regulators phospho-AKT and BCL-X\textsubscript{a}, as well as increases in the pro-apoptotic markers BAK and PARP cleavage. Hallmark features of HDAC inhibition, including up-regulation of cyclin-dependent kinase p21 and hyperacetylation of histone H3 were also observed. In vivo efficacy of AR-42 was demonstrated
in a subcutaneous AsPC-1 tumor xenograft and a transgenic (LSL-Kras$^{G12D}$;P53$^{flox/flox}$;Pdx-1-cre(+)) mouse model of pancreatic cancer. Mice were treated with AR-42 at 50mg/kg by oral gavage every other day, resulting in suppression of tumor burden in xenograft and transgenic models by 57% and 42%, respectively. Tumor suppression in AsPC-1 xenograft tumors was associated with HDAC inhibition, increased apoptosis, and inhibition of proliferation. These results suggest that use of AR-42 represents a therapeutically promising strategy for the suppression of tumor growth in pancreatic cancer. Future in vivo studies will test AR-42 in combination with the standard of care gemcitabine on primary and metastatic tumor burdens and survival.

**Poster Number:** E-23

**Section:** Experimental Disease  
**Keyword:** Neoplasia

**NLRX1 ATTENUATES TUMORIGENESIS THROUGH THE NEGATIVE REGULATION OF AKT AND NF-κB SIGNALING**

S. Coutermarsh-Ott, T. LeRoith, C. Washington, N. Dervisis, R. Hontecillas-Magarzo, J. Bassaganya-Riera, and I. Allen  
Virginia Tech, VA-MD College of Veterinary Medicine, Dept. of Biological Sciences and Pathobiology, Blacksburg, VA, Virginia Tech, Virginia Bioinformatics Institute, Nutritional Immunology and Molecular Medicine Laboratory, Blacksburg, VA

Histiocytic sarcoma (HS) is a rare to uncommon, malignant neoplasm with a phenotypic profile consistent with an interstitial dendritic cell or macrophage origin. In veterinary medicine, it is primarily a canine disease though few cases have been identified in cats. Burnese mountain dogs, Flat-coated retrievers and Golden retrievers tend to be overrepresented, however, histiocytic sarcoma has been identified in a variety of other breeds. Treatment options in both human and veterinary medicine are limited and the disease is often fatal. In our lab, we are interested in investigating the role of NLRX1 in tumorigenesis. NLRX1 is a recently characterized, intracellular pattern recognition receptor involved in the negative regulation of inflammation. We hypothesized that it is also involved in the negative regulation of tumorigenesis. To test this hypothesis, we utilized the well-established model of urethane-induced tumorigenesis. Mice lacking NLRX1 that were exposed to urethane developed increased morbidity and mortality early in the study due to the development of histiocytic sarcoma. Mechanistically, our data suggest that NLRX1 attenuates tumorigenesis through the negative regulation of AKT and NF-κB signaling. Together, this data extends the currently characterized function of NLRX1 as well as reveals new pathways involved in the pathogenesis of histiocytic sarcoma.

**Poster Number:** E-24

**Section:** Experimental Disease  
**Keyword:** Immune System

**TRANSFORMING GROWTH FACTOR BETA INHIBITION REDUCES OSTEOSARCOMA PULMONARY METASTASES**
Transforming growth factor beta (TGF beta) is a pleotropic cytokine which regulates multiple homeostatic pathways including bone morphogenesis, immunity, and angiogenesis. Circulating TGF beta 1 concentrations correlate with neoplastic disease burden and have also been implicated in facilitating metastasis. In dogs and people with spontaneous osteosarcoma (OS), mortality often results from the establishment and progression of pulmonary metastases. The purpose of this investigation was to determine if TGF beta signaling participates in OS metastatic progression, and if inhibiting the TGF beta signaling pathway could reduce pulmonary metastatic burden. Through the use of a relevant preclinical murine model of OS (K7M2 cell line), the biologic effects of saline (control), 1D11 (pan-blocking TGF beta signaling antibody), or 13C4 (an irrelevant isotype control antibody) administered intraperitoneally every other day were studied for their capacity to attenuate experimental pulmonary metastases formation. Cell proliferation, survival, angiogenesis, and cytokine profiling assays were conducted, as well as organ histopathology. Six administrations of 1D11 given over 2 weeks significantly reduced the number and size of pulmonary metastases by 50% compared to mice receiving saline or 13C4. This reduction in pulmonary metastases was not mechanistically linked with cell proliferation (Ki-67 score), angiogenesis (circulating VEGF), or inflammatory cytokines, but rather anoikis. No histopathological evidence of autoimmunity was identified in any group. Inhibiting the TGF beta pathway reduces the establishment of pulmonary metastases through mechanisms likely associated with cell survival. Small molecule inhibitors of TGF beta signaling might be a suitable adjunct therapy for improving treatment outcomes in dogs with OS.

**Poster Number:** E-25

**Section:** Experimental Disease  
**Keyword:** Neoplasia

**ACTIVATION OF K-rasG12D IN K-rasLSLErGFPcre MOUSE MODEL LEADS TO HEMATOPOIETIC, VASCULAR AND EPITHELIAL DEFECTS**

S.R. Jayapal, M. Al-Haddawi, C.Q. Wang, F.M. Ibrahim, M. Osato, and P. Kaldis  
1Institute of Molecular and Cell Biology, A*STAR, Republic of Singapore, 2Cancer Science Institute of Singapore, National University of Singapore, Republic of Singapore

K-ras is a member of the Ras family of small GTPases that are expressed in all cell types and are critical regulators of intracellular signalling pathways controlling normal development and oncogenesis. K-ras mutations have been associated with a variety of cancers and developmental diseases such as acute myeloid leukemia, myelodysplastic syndromes, myeloproliferative disorders, gastric adenocarcinoma, colon cancer, lung adenocarcinoma, vascular neoplasm, cardiac rhabdomyosarcoma and angiosarcoma, liver angiosarcoma, gastric hyperplasia and metaplasia, and lymphatic vessel abnormalities.

In this study, K-ras<sup>LSL</sup> mice were crossed with ErGFPcre (Erythropoietin receptor promoter-driven Cre) mice to obtain K-ras<sup>LSL</sup>ErGFPcre mice, which expressed K-ras<sup>G12D</sup> in cells that express the erythropoietin
receptor. These mice were born at frequencies lower than expected Mendelian ratios, appeared overtly normal, and died around 3 months of age. Expression of K-rasG12D resulted in activation of MAPK (ERK1/2) pathway associated with microscopic findings of increased extramedullary hematopoiesis in spleen and multiple lymph nodes, vasculature (lymphatics and blood vessels) abnormalities in skin, liver, heart, tongue and lung, as well as epithelial hyperplasia in stomach and thyroid. The histopathological findings in this K-ras activated mouse model resemble precancerous lesions. Therefore, the present model of K-rasG12D activation could be used to study further the development and progression of blood, vascular, and epithelial carcinogenesis.

**Poster Number:** E-26

**Section:** Experimental Disease  
**Keyword:** Neoplasia

**SPONTANEOUS TUMORS IN CHIMERIC RATS GENERATED FROM DARK AGOUTI ES CELLS**

K. Yekkala¹, J.M. McGrath², T. Nottoli², Y. Yang², and C.J. Zeiss²  
¹Toxikon Corporation, Bedford, MA, ²Dept. of Comparative Medicine, Yale University School of Medicine, New Haven, CT

This study investigated spontaneous lesions and cause of death in a series of 7 chimeric rats generated from dark Agouti ES cells. 3/7 ES cell derived rats and one offspring had concurrent lymphoma and hemangiosarcoma, and one animal experiencing hemangiosarcoma only. Gross lesions in 5/7 rats included splenic rupture with hematoma. Microscopically animals with concurrent tumors had marked extramedullary hematopoiesis, extensive axial and appendicular skeletal invasion by neoplastic lymphocytes, severe osteoclastic hyperplasia and bone resorption, and pathological fractures along with axonal degeneration of cervical, thoracic and lumbar spinal cord. In one animal there was liver and heart metastasis of hemangiosarcoma with extensive necrosis. Neoplastic lymphocytes were Pax5 positive, CD3, B220 and CD68 negative indicating a pre-B cell lineage and neoplastic endothelial cells were factor 8 related antigen positive. Complete blood count revealed that 2/7 animals were leukemic (Pax5 positive lymphoblasts) and one animal exhibited monoclonal gammopathy on urine electrophoresis. Based on the gross and histopathological lesions, immunohistochemistry, extensive osteoclastic proliferation and bone resorption, monoclonal gammopathy on urine electrophoresis, the disease in these chimeric rats was diagnosed as B cell lymphoma with features of multiple myeloma. Coexistence of both tumors across generations implies their transmission as a Mendelian trait, most likely resulting from a cytogenetic lesion or a spontaneous mutation in the source ES cells. Due to a short clinical duration and features of malignancy, a potential for using our chimeric rats as a model for studying molecular mechanism of multiple myeloma and potential therapeutic intervention is currently under investigation.

**Poster Number:** E-27

**Section:** Experimental Disease  
**Keyword:** Neoplasia
DETERMINING THE ROLE OF POST-CASTRATION INFLAMMATION IN THE PROGRESSION OF PROSTATE CANCER USING A SYNGENEIC MOUSE MODEL

C.J. Pinelli, S.D. Kim, and G.A. Wood
Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON

Inflammation has been linked to the development and progression of a number of different cancers, including prostate cancer. Tissue inhibitor of metalloproteinase-3 (TIMP3) is an endogenous inhibitor of inflammation, specifically through inhibition of matrix metalloproteases (MMPs) and other enzymes, and its loss has been linked to prostate cancer development. Our lab previously used Timp3 knockout (KO) mice crossed into prostate-specific Pten conditional knockout mice to investigate the role of Timp3 loss in prostate cancer, and found increased inflammation and MMP activity with shorter tumor latency in Timp3 KO prostate tumours compared to wildtype (WT). The objective of this study was to determine if this finding is consistent across other models of prostate cancer and if post-castration prostatic inflammation can accelerate tumour progression. RM cells are murine prostate cancer cells on a C57BL/6 background derived from fetal urogenital sinus cells overexpressing Myc and Ras oncogenes. These syngeneic cells were injected orthotopically into the left dorsolateral lobe of the prostate of Timp3 WT and KO mice, and allowed to grow for 2 weeks, at which point mice were castrated. Preliminary data shows that TIMP3 KO mice injected with androgen independent RM9 cells have larger tumours with worsening clinical condition at earlier time points post-castration compared to WT mice. These findings are similar to those in our previous mouse model and further support the role of inflammation and MMP activity in progression of castration resistant prostate cancer. Additional analyses (histology, immunohistochemistry and protein quantification) are being performed to characterize the associated inflammatory infiltrate.

**Poster Number:** E-28

**Section:** Experimental Disease

**Keyword:** Neoplasia

AURORA KINASE INHIBITION IN EWING SARCOMA XENOGRAFTS

A.C. McCalla\(^1,2,3,5\), N.J. Caplen\(^4,5\), and L.J. Helman\(^1,5\)

\(^1\)Pediatric Oncology Branch, \(^2\)NIH Comparative Biomedical Scientist Training Program, \(^3\)College of Veterinary Medicine, North Carolina State University, \(^4\)Genetics Branch, \(^5\)Center for Cancer Research, National Cancer Institute, NIH Bethesda, MD

Ewing sarcoma (ES) is a pediatric bone tumor that most often occurs as a consequence of an 11:22(q24:q12) chromosomal translocation. This translocation forms a fusion oncogene encoding the transcription factor EWS-FLI1 that deregulates >500 gene targets. Two genes overexpressed in ES encode the Aurora kinase mitotic regulators, AURKA and AURKB. Previous work has shown ES cells are sensitive to AURK inhibition *in vitro*. In this study, ES xenograft models were assessed following inhibition of AURKA and AURKB *in vivo*. Xenografts were generated in SCID mice by injecting ES cells into the gastrocnemius muscle. Mice were randomized into “treatment” and “vehicle” groups when tumors reached 200-400mm\(^3\) or >2000mm\(^3\) (end-stage) for tumor growth inhibition (TGI) or tumor regression
(TR) studies, respectively. Groups received either the AURKA inhibitor Alisertib, the AURKB inhibitor Barasertib, or a vehicle treatment. Alisertib treated mice (15) showed no TGI effects as compared to vehicle treated mice (15) in the TC32 xenografts and was not tested further. In contrast, complete TGI (15/15) and TR (10/10) was attained in the Barasertib treated TC32 xenografts within one week of treatment. Resistance was attained after five weeks of treatment in 11/15 mice. Barasertib treatment of EW8, TC71 and RDES xenografts resulted in a 100, 88, and 66% reduction in tumor doubling size, respectively, over a ten day period (p<0.05), but had no effect on TR. Collectively, our findings indicate AURKB is an ideal target for development of future ES therapeutic strategies, yet a combination approach may be needed for retained efficacy.

Poster Number: E-29

Section: Experimental Disease
Keyword: Neoplasia

HUMAN-LIKE HISTOLOGY IN A UV-INDUCED BRAF V600E MUTANT MOUSE MODEL OF MELANOMA

H. Michael, C-P Day, and G. Merlino
National Cancer Institute, Bethesda, MD

Malignant melanoma is an aggressive and deadly neoplasia in people and the number of cases has been increasing over the last few decades. Benign melanocytic nevi are ubiquitous in the population and rarely can progress to malignant melanoma decades later. Activation of the MAP kinase pathway is common, and BRAFV600E mutations are found 90% of nevi and 50% of melanomas. The exact role of BRAFV600E mutation remains unclear, but is thought to be an early event. Therapies targeting BRAFV600E mutations lead to marked short-term clinical improvement, followed by inevitable relapses. Without genetic engineering, mice have hair follicle-restricted melanocytes and are resistant to formation of melanoma. Current mouse model of BRAF-mutant melanoma are often coupled with additional driver mutations, are mostly non-UV initiated, and form primarily dermal tumors with fairly homogenous histology. Our hepatocyte growth factor (HGF) mouse model has a “humanized” junctional distribution of melanocytes and develops melanocytic lesions following neonatal UV exposure. HGF+-CreERTA-Tyr Ca/+ BRAFV600E Ca/+ CDKN2A fl/+ mice are exposed to neonatal UV followed by induction of the BRAFV600E mutation with tamoxifen at 4 weeks. Treated mice develop melanomas that recapitulate the histologic variability of BRAF mutant human melanoma, including junctional origin and variable immune infiltration. The HGF-BRAF mutant model is a powerful new tool for investigating the role of BRAF and UV in the development and progression of melanocytic nevi and melanoma. Using this powerful new model could provide important information about carcinogenesis and progression that could inform clinical management of BRAF V600E mutant human melanoma.

§ Poster Number: E-30

Section: Experimental Disease
Keyword: Neoplasia
QUANTIFICATION OF TELOMERASE IN AN ORAL SQUAMOUS CELL CARCINOMA MODEL

W. Supsavhad, W. Dirksen, and T. Rosol

Telomerase is a novel target for human cancer therapies, and telomerase inhibitors are being used in four phase II and ten phase I clinical trials. We have developed a feline model of oral squamous cell carcinoma (OSCC) using cell lines and nude mice that mimics the human condition and can be used for translational research. We investigated the levels of telomerase activity and mRNA expression in feline OSCC model. Human and feline telomerase catalytic subunit (TERT) and telomerase RNA component (TERC) sequences were aligned revealing a high degree of similarity. Telomerase activity was measured with a telomerase detection assay and fTERT mRNA expression was quantitated by real-time RT-PCR in three feline OSCC cell lines, normal oral tissues, and three feline frozen OSCC biopsies. Telomerase activity and fTERT mRNA expression in the three OSCC cell lines were significantly greater than normal cat oral tissues. Telomerase activity in 2 of 3 frozen OSCC samples was also significantly higher than normal oral tissues. In conclusion, telomerase activity and fTERT mRNA expression in feline OSCC were comparable with human cancer cells. Ongoing studies include measuring the effect of an oligonucleotide antisense telomerase inhibitor on telomerase activity, cell morphology, growth, viability and apoptosis in feline OSCC cell lines as well as immortalization of telomerase-negative feline mesenchymal stem cells (MSC) with cloned feline telomerase.

Poster Number: E-31

Section: Experimental Disease
Keyword: Reproductive System

EXPRESSION OF STEM CELL AND EPITHELIAL-MESENCHYMAL TRANSITION (EMT) MARKERS IN PROGRESSION OF HUMAN CERVICAL NEOPLASIA AND FOLLOWING CIGARETTE SMOKE CONDENSATE EXPOSURE IN HUMAN ECTOCERVICAL CELLS

X. Gao, C. Fulbright, L. Yu, L. Castro, D. Walmer, and D. Dixon

Smoking is a critical risk factor in cervical carcinogenesis. Though the mechanisms underlying smoking’s carcinogenic effects are unclear, preliminary studies have found that cigarette smoke initiates an early epithelial-mesenchymal transition (EMT) in vitro. Certain EMT activators can co-induce EMT and “stemness” properties. In this study, human ectocervical (Ect1/E6E7) cells treated with 10 mg/mL cigarette smoke condensate (CSC) and human cervical tissue samples were used to explore the potential effects of CSC on the pathogenesis of cervical cancer. Cells treated with CSC lost their “cobblestone” morphology and became “spindle-like” after 3 days. Using real-time PCR, expression of EMT biomarkers, FSP1, MMP9, Snail1, Twist1 and vimentin were up-regulated, while E-cadherin gene expression was down-regulated following exposure to CSC for 1, 3, and 90 days. The expression of CD44v6, a marker of cancer stem cells, was increased following 90 days of CSC exposure by western blot analysis. Immunofluorescence studies showed a significant increase in CD44v6 signals in CSC-exposed cells at 90 days. Additionally, CD44v6 protein expression in human cervical tissue samples, ranging from cervical
intraepithelial neoplasia (CIN) grade I to squamous cell carcinoma, was increased as CIN grade progressed to cervical cancer. Our data suggest that CSC-induced EMT cells may have the potential to turn into aberrant stem cells, or cancer stem cells, due to their expression of a stem cell marker that is increased as CIN grade progresses to cervical cancer in human tissue samples. These findings suggest a potential mechanism explaining smoking’s carcinogenic outcomes in the cervix.

Poster Number: E-32

Section: Experimental Disease Focused Scientific Session I

Keyword: Neoplasia

GENOME WIDE IDENTIFICATION OF SUSCEPTIBILITY LOCI FOR MALIGNANT PHENOTYPES IN PULMONARY ADENOCARCINOMA

E.F. Edmondson\(^1,2\), D.M. Gatti\(^3\), C.M. Fallgren\(^2\), R.R. Rampersad\(^4\), and M.M. Weil\(^2\)

\(^1\)Microbiology, Immunology, Pathology, \(^2\)Environmental and Radiological Health Sciences

Departments, Colorado State University, Colorado, \(^3\)The Jackson Laboratory, BarHarbor, Maine,

\(^4\)Department of Surgery, Duke Medicine, Durham, North Carolina, USA

Pulmonary sarcomatoid carcinomas (PSCs) are a subset of non-small cell lung carcinomas with an aggressive clinical course and a high rate of metastasis. The sarcomatoid component of PSCs is thought to arise as the result of epithelial-mesenchymal transition (EMT) during carcinogenesis progression. We have identified a model for PSC in a genetically heterogenous population of mice. Of 349 mice with pulmonary adenocarcinoma, 28 developed PSC (10 of which metastasized). Metastatic disease was significantly more common in mice with PSC (p<0.001). To verify that the sarcomatoid component of these tumors indeed arose from pulmonary adenocarcinoma cells, a subset of poorly differentiated PSCs were analyzed for Club Cell antigen and Surfactant protein C. To determine how host genetic variation contributes to the development of this trait, each mouse was genotyped using dense SNP arrays for QTL mapping. Three QTL were identified which were associated with sarcomatoid differentiation and distant metastasis. One locus on chromosome 1 was narrow and contained a single protein coding gene: Prox1. Although overexpression of PROX1 has been shown to promote EMT in colon cancer, germline polymorphisms in Prox1 that control the susceptibility to EMT have not been identified. Analysis of SNPs in Prox1 between the eight founders used to create our mouse model identifies multiple SNPs in the 3'UTR of the mRNA product. These results identify multiple QTLs associated with the development of PSC and metastasis and indicate that alteration in PROX1 expression is a likely event during epithelial-mesenchymal transition and metastasis in pulmonary adenocarcinomas.

Poster Number: E-33

Section: Experimental Disease

Keyword: Neoplasia

PROSTATE INFLAMMATION INCREASES CARCINOMA DEVELOPMENT IN THE POET3+PTEN-/- MOUSE MODEL OF PROSTATE CARCINOGENESIS
Inflammation has been associated with enhanced carcinogenesis in multiple organs; however, the relationship between prostatitis and prostate cancer development is not well understood. The previously-described POET-3 mouse model of autoimmune prostatitis was crossed with the C57/Luc/Pten−/− mouse model of prostate carcinogenesis, which utilizes the Cre-loxP system driven by the prostate-specific Probasin promoter to inactivate both alleles of Pten in the prostate. Using this model, multiple episodes of prostate-specific inflammation were induced and prostates were examined for lesion development, leukocytic infiltration, and cytokine expression. Mice in both groups developed severe prostate inflammation, starting as early as 8 weeks of age, with mice in the induced inflammation group showing an increased proportion of CD8+ T cells. Young mice with previous bouts of inflammation showed significantly more carcinoma development than age-matched mice without induced inflammation, with this observation continuing as a non-significant trend later in life. Mice at 26 weeks of age with previous episodes of inflammation showed increased amounts of CD8+ and CD4+ T cells, but no differences were observed in leukocytic infiltrate in mice that developed carcinoma versus those that did not. No differences in expression levels of various cytokines were observed between mice with previous episodes of inflammation or those treated with vehicle controls. Taken with previous results showing that induced prostatitis had no effect on carcinoma development in POET3+/−Pten−/− mice, these data suggest that inflammation has a greater effect on prostate carcinogenesis in the context of complete loss of Pten.

**Poster Number:** E-34  
**Section:** Experimental Disease  
**Keyword:** Neoplasia

**AH R DELETION INCREASES RATE OF THYMIC LYMPHOMA AND DECREASES SURVIVAL IN P53-DEFICIENT MICE**

J.L. Phillips, C.V. Löhr, and S.K. Kolluri  
Oregon State University, Corvallis, OR

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates a diverse set of genes. Although the AhR is best known as regulator of drug-metabolizing enzymes, particularly with respect to induction of AhR by the ligands 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other polycyclic aromatic hydrocarbons (PAHs), research indicates that the AhR could also mediate the beneficial effects of drugs used to treat cancer. While much is known about the consequences of AhR activation by exogenous ligands, little is known about the endogenous functions of the receptor and its role in suppressing or driving tumor formation. We hypothesized that the AhR functions to suppress
tumorigenesis, as suggested in previous studies, and studied the role of the AhR as a tumor suppressor in the absence of p53.

We developed an AhR-knockout mouse model lacking the tumor suppressor protein p53, mimicking the most common mutation evident in the development of human cancers. The absence of AhR increased the frequency of thymic lymphoma and reduces survival in p53-null mice, suggesting a role for the AhR in suppressing tumor formation. Mice lacking both p53 and AhR also showed non-tumor phenotypes of otitis and gastritis, were generated at a lower frequency than predicted, and were of smaller size than their respective control littermates. In summary, the absence of AhR in p53-null mice results in reduced survival, increased rate of thymic lymphoma development, and is associated with reduced embryonic survival and neonatal fitness. These findings suggest important roles for the AhR in tumor suppression and development.

**Poster Number:** E-35

**Section:** Experimental Disease  
**Keyword:** Immune System

**CONJUGATED LINOLEIC ACID IN THE TREATMENT OF MURINE AUTOIMMUNE DISEASE**

S. Barrett, N. Regna, A. Gojmerac, C.Chafin, M. Vieson, and C. Reilly

Conjugated linoleic acid (CLA) has been shown to reduce inflammation via Peroxisome Proliferator-Activated Receptor (PPAR)-γ in inflammatory disorders. We sought to determine whether CLA isomers would reduce inflammation in cultured mesangial cells, and in murine autoimmune glomerulonephritis. Mesangial cells were cultured with pure CLA isomers (c9,t11 or t10,c12) or a 50:50 mixture prior to immune stimulation. Next, cultured mesangial cells were transfected with siRNA targeting PPARγ and treated with CLA isomers prior to immune stimulation. ELISA, RT-qPCR and Western blot were performed to measure cytokine production, mRNA expression, and iNOS production. Treatment with c9,t11 CLA reduced IL-6 production in cultured mesangial cells, but not in siRNA-treated mesangial cells, suggesting c9,t11 CLA acts through PPARγ. Conversely, t10,c12 CLA failed to reduce IL-6 and iNOS production in cultured mesangial cells. For in vivo studies, mice which spontaneously develop autoimmune disease (NZM2410/J) were fed a control diet or a c9,t11-CLA-supplemented diet beginning at 10 weeks of age. Treatment with CLA failed to reduce or prevent disease. Moreover, mice receiving CLA had both reduced survival times and increased renal inflammation relative to untreated controls. Taken together, these studies suggest that while CLA treatment reduces inflammatory mediator production in mesangial cells in vitro, it does not reduce morbidity and mortality in vivo, and may exacerbate inflammation in murine autoimmune disease.

**Poster Number:** E-36

**Section:** Experimental Disease  
**Keyword:** Immune System
TREATMENT WITH A SELECTIVE HISTONE DEACETYLASE 6 INHIBITOR DECREASES LUPUS NEPHRITIS IN NZB/W MICE

M.D. Vieson¹, A. Gojmerac², D. Caudell², S.H. Barrett¹, and C.M. Reilly⁴
¹Dept of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, ²Virginia Polytechnic and State University, Blacksburg, VA, ³Dept of Pathobiology/Comparative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, ⁴Edward Via College of Osteopathic Medicine, Blacksburg, VA

To date, there are 17 histone deacetylase (HDAC) enzymes, divided into 4 classes, which alter protein function by removing acetyl groups from lysine residues. Prior studies report that non-selective HDAC inhibitors decrease disease in various lupus mouse models. Selective HDAC-6 inhibition acts on cytosolic proteins to decrease B cell proliferation and differentiation by inhibiting α-tubulin function. Since B cells play a critical role in the initiation and propagation of systemic lupus erythematosus, we hypothesized that a selective HDAC-6 inhibitor (HDAC6i) will alleviate disease in a mouse model. Intraperitoneal injections of HDAC6i (0.3 mg/kg, 1 mg/kg, or 3 mg/kg), vehicle control, or dexamethasone were administered to 21-week-old, female NZB/W mice, 5 days a week, for 13 weeks. Disease was evaluated by body weight, proteinuria, serum levels of anti-dsDNA antibody, cytokines and immunoglobulins, and post mortem evaluation of nephritis and B cell populations in the bone marrow and spleen. Treatment with HDAC6i decreased glomerular scores, spleen weights, and urine protein scores when compared to vehicle-treated mice. No differences in B cell development and differentiation in the bone marrow, and B cell activation in the spleen were noted. We conclude that HDAC-6 inhibitors are effective at decreasing lupus nephritis and disease in NZB/W mice, however further studies are warranted to investigate the underlying mechanism, particularly in regards to B cell pathophysiology.

Poster Number: E-37

Section: Experimental Disease Focused Scientific Session I
Keyword: Alimentary

LOSS OF NLRX1 RESULTS IN INCREASED INTESTINAL PATHOLOGY AND EXACERBATED T CELL RESPONSES IN MICE WITH INFLAMMATORY BOWEL DISEASE

K. Eden¹,³, R. Hontecillas¹, M. Viladomiu¹, C. Philipson¹, A. Carbo¹, A. Leber¹, N. Philipson¹, I. Tattoli³, S.E. Girardin², I.C. Allen³, and J. Bassaganya-Riera¹,³
¹Nutritional Immunology and Molecular Medicine Laboratory, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, ²Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, ³Department of Biomedical and Veterinary Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

NLRX1 is a mitochondrial-associated NOD-like receptor that modulates antiviral immunity, cellular stress, autophagy, and reactive oxygen species (ROS) production. The role of NLRX1 in inflammatory bowel disease (IBD) remains largely unknown. We investigated the ability of NLRX1 to modulate gut pathology, inflammation, and immunity in both acute and chronic mouse models of IBD by using dextran
sodium sulfate, CD4+CD45RB^{hi}CD25^{-} adoptive transfer, and CD4+CD45RB^{hi}/CD4+CD45RB^{lo}CD25^{+} cotransfer colitis. Colon, spleen, and mesenteric lymph nodes (MLN) were excised for immunophenotyping via flow cytometry, microscopic lesion formation, gene expression, ROS production, and protein analysis. The loss of NLRX1 both globally and specifically in CD4+ T cells increased severity of disease and colonic histopathology, including increased leukocytic infiltration, proliferation, fibrosis, and crypt abscessation in both colon and ileum. Numbers of effector Th1, Th17, and regulatory T cells (Treg) in the colonic mucosa, mesenteric lymph nodes, and spleens were also dramatically altered due to NLRX1 deficiency in both models. Loss of NLRX1 also resulted in increased colonic ROS production and and upregulated downstream cytokines IL-17, IFNγ, TNF-α, and autophagy-related proteins in the acute and chronic models, respectively. Cotransfer experiments revealed that the lack of NLRX1 specifically in effector T cells resulted in increased disease pathology; NLRX1/-/ Tregulatory cells appeared to retain some functionality. We present NLRX1 as an important new player in the pathology and immunology of murine models of inflammatory bowel disease.

**Poster Number:** E-38

**Section:** Experimental Disease  
**Keyword:** Alimentary

**IL-27 ACTS ON EPITHELIAL AND INFLAMMATORY CELLS IN THE RESOLUTION OF MURINE COLITIS**

C. Andrews, M. McLean, M.L. Hanson, and S.K. Durum  
Cancer and Inflammation Program, National Cancer Institute, Frederick, MD

Inflammatory bowel disease (IBD) represents a collection of inflammatory disorders of the intestine thought to result from inappropriate exposure of resident intestinal immune cells to luminal antigens due to compromise of the epithelial barrier. In humans and animals, IBD treatment typically involves systemic immunosuppression, necessitating a balance between achieving remission and managing adverse treatment effects. As a result, new therapies for IBD are needed. Intestinal mucosal administration of interleukin-27 (IL-27) synthesized in situ by the food-grade bacterium *Lactococcus lactis* reduces disease activity and colon pathology in three murine colitis models, including enterocolitis induced by T cell transfer and two chemically-induced colitis models. While the therapeutic effects of IL-27 depend on IL-10, the requirement for T cells varies between models, making it unclear which cell types directly respond to IL-27. In this study we demonstrate that mucosally-delivered IL-27 (LL-IL-27) activates superficial and crypt epithelial cells, as well as infiltrating macrophages and neutrophils within the mucosa and submucosa, as evidenced by STAT1 phosphorylation and nuclear translocation. Interestingly, concurrent intestinal inflammation was required for LL-IL-27-induced STAT1 activation, which was largely absent in healthy mice gavaged with LL-IL-27. Similarly, LL-IL-27 treatment following chemical induction of colitis reduced colon pathology, while LL-IL-27 was inefficacious as a prophylactic treatment. These findings provide crucial insight into the mechanism of IL-27 treatment in colitis and inform the proper timing of this treatment in future clinical trials. Additionally, these data underscore the ability of IL-27 to both modulate inflammation and directly act upon epithelial cells in intestinal inflammation.

**Poster Number:** E-39
THE EFFECT OF PI3K GAMMA KD AND PI3K DELTA KD ON INTESTINAL LESIONS IN MICE WITH DSS-INDUCED COLITIS

1University of Pennsylvania, College of Veterinary Medicine, 2Janssen Research and Development, Spring House, PA, 3Janssen Research and Development, La Jolla, CA

The Phosphoinositide-3-Kinases (PI3K) family of lipid kinases have been a popular target for the treatment of some cancers and autoimmune diseases such as colitis. Of particular interest are the roles of PI3K Delta and PI3K Gamma. Current literature claims that the inhibition of PI3K Gamma or genetic silencing of kinase activity can ameliorate Dextran Sulfate Sodium (DSS) induced colitis by attenuating leukocyte infiltration and by immunoregulating the imbalance between pro-inflammatory and anti-inflammatory cytokines. In contrast, mice harboring inactive PI3K Delta showed a propensity for an increased incidence of colitis that is age-related and microbiota-dependent. To further elucidate the roles of PI3K Delta and PI3K Gamma in the mucosal immune response, we examined DSS colitis in C57BL/6 Wild-Type mice, PI3K Delta kinase-dead mice and PI3K Gamma kinase-dead (KD) mice. Mice (5-8 weeks; male/female) were treated with 3% DSS in water ad lib for 4 days followed by 2 days of acidified drinking water (pH 2.4-2.5), and humanely sacrificed. Colon lengths, body weights, and histopathology were primary endpoints. Histologic sections of the proximal and distal colon were examined and graded on a 4 point scale (minimal, mild, moderate, or marked) for inflammation, glandular necrosis/dropout, edema, and mucosal erosion/ulceration. These histological lesions associated with DSS-induced colitis were of a decreased incidence and severity in the PI3K Delta KD mice when compared to the Wild-Type and PI3K Gamma KD, which had similar lesion profiles. The colon length results showed no significant differences when the wild-type, PI3K Delta KD, and PI3K Gamma KD were compared.

Poster Number: E-40

Section: Experimental Disease
Keyword: Nervous System

A METHOD FOR HISTOPATHOLOGIC STUDY OF THE MULTIFOCAL NATURE OF SPINAL CORD LESIONS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALITIS

Department of Pathology, Roy J. & Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA
Experimental autoimmune encephalitis (EAE) is an established mouse model for multiple sclerosis and is characterized by immune cell infiltration, primarily T cells and macrophages, within the central nervous system. EAE is a multifocal and random disease, which makes histopathologic analysis of lesions difficult as it is impossible to predict where lesions will occur within the brain and spinal cord, especially when evaluating cross sections of spinal cord. To histologically evaluate the entire length of the spinal cord in EAE we have developed a method to section the cord within the decalcified spinal column which allows for the study of the multifocal nature of this disease. HE and Luxol fast blue staining of longitudinally sections spinal cord reveal a paucity of lesions while in other areas there is marked inflammation and demyelination. The percentage of spinal cord affected by EAE was evaluated at seven separate areas of the longitudinally sectioned HE-stained cord and it varied greatly within a single sample. Immunohistochemical staining of these decalcified cords was successful for key immuno-markers used in EAE research including CD3 for T cells, B220 for B cells and F4/80 for murine macrophages. This method of histopathologic analysis will allow EAE investigators to look at the entire spinal cord on a single slide and evaluate areas of the spinal cord with and without classic EAE lesions.

Poster Number: E-41

Section: Experimental Disease
Keyword: Cardiovascular

VASCULAR LUMEN PRESERVATION AND OPTIMIZATION FOR IN VIVO-LIKE PERIPHERAL VASCULATURE DIMENSIONS IN HISTOLOGY FOR PROPER PRECLINICAL DEVICE EVALUATION

A. Tellez, K.N. Dillon, J.A. Wicks, and S.D. Rousselle

Objective: Elastic arteries, following harvesting, experience pronounced radial and longitudinal recoil. We aimed to establish an innovative methodology with the intent of preserving the in vivo vascular dimensions in histology.

Methods: Peripheral arteries of 7 Yucatan naïve swine were evaluated. Arteries were flushed and pressure fixated. While still in situ, some vessels were infused with a polymeric-based vascular intraluminal support. Vessels were evaluated by histology and histomorphometry and compared to equivalent angiographic data. Following randomization, 10 arteries were fixed with 10%NBF (UIV) and 18 arteries were preserved with the vascular intraluminal support (VIS).

Results: When VIS was utilized (External Iliac Artery [EIA]=3.77±0.61mm;Superficial femoral artery [SFA]=3.3±1.26mm), the lumen diameters were preserved compared to angiography (EIA=4.87±0.46mm;SFA=3.63±0.34mm), whereas UIV SFAs showed a significant decrease (EIA=2.07±0.56mm;SFA=1.94±0.36mm). Histomorphometry showed a reduction in lumen area of EIA (VIS=11.49±3.5mm²;UIV=3.83±1.6mm²) and SFA (VIS=6.99±1.8mm²;UIV,3.09±1.2mm²). Vessel area followed the same pattern (EIA[VIS,12.9±3.6mm²;UIV,5.9±1.5mm²]; SFA[VIS,8.06±1.9mm²;UIV,4.83±1.1mm²]). In the absence of VIS, both EIA and SFA showed an artificially thicker vascular wall (EIA=249.18±87.7μm;SFA=243.15±87.7μm) compared to VIS group (EIA=117.46±25.2μm;SFA=108.75±20.6μm). UIV displayed prototypical features such as collapse profile, ‘zig-zags’ internal elastic lamina (IEL) pattern, thick media, and thick and corrugated external elastic
lamina (EEL). In contrast, VIS showed a circular profile, stretched and curvilinear IEL and EEL, and a thin media layer with concentrically layered streams of smooth muscle cells. In both groups vessels had a continuous and confluent endothelium.

Conclusion: In relevant preclinical studies where luminal support is lacking it is of paramount importance to implement this novel histology paradigm.

**Poster Number:** E-42

**Section:** Experimental Disease  
**Keyword:** Cardiovascular

**CELL PROLIFERATION MARKERS AS A VALUABLE TOOL FOR PRECLINICAL EVALUATION OF VASCULAR INTERVENTIONS**

K. Dillon, S. Wilson, J. Guerrero, D. Brady, W. Grundy, J. Wicks, S. Rousselle, and A. Tellez  
Alizée Pathology, Thurmont, MD, MPI Research, Mattawan, MI, Celladon Corporation, San Diego, CA

**Introduction:** Patency and maturity of an arteriovenous fistula (AVF) are of primary importance. However, AVF dysfunction caused by venous intimal hyperplasia persists as a significant threat. In addition to conventional vascular planimetry we aim to determine cell proliferation in a swine model of AVF.

**Methods:** Femoral AVF were surgically performed in nine swine by anastomosis. AVFs were followed for 3(n=3) and 90(n=6) days. At termination, angiography was performed to verify patency. Following the harvesting of the AVFs, planimetric analysis was performed. Immunohistochemistry for cellular proliferation (Ki67) was performed at the anastomosis levels as well as the arterial (A) and venous (V) anastomosis side.

**Results:** At 3 days, AVF displayed matching dimensions between V and A with a vascular area of 13.2±4.3 mm² in A and 15.1±9.4 mm² in V. By 90 days, the vascular area on V increased by approximately 4-fold (58.6±42.5 mm²) while A increased by less than 3-fold (37.3±9.4 mm²). These planimetric ratios remained consistent in the proximal A and distal V at 3(A=14.3±4.1 mm²; V=36.8±14.8 mm²) and 90 days (A=40.2±12.8 mm²; V=68.6±28.2 mm²). At 3 days, percent area of stenosis (%AS) at the anastomosis was minimal (A=1.8±1.5%; V=5±6.1%). However at 90 days, V demonstrated markedly increased %AS (21.9±16.5%) compared to A (6.9±2.9%), reflecting V being arterialized. As expected, cell proliferation counts were high at 3 days evidenced by a high Ki67 index in V (159.2±134.7cells/mm²) and highest values were recorded in the V distal outflow side. Ki67 counts significantly decreased at 90 days (35.2±22.4cells/mm²).

**Conclusion:** Proliferation markers (Ki67) provide valuable assessment of vascular response. The AVF swine model provides an ideal scenario where conventional planimetry data describes the structural outcome while proliferative markers provide a dynamic picture of the biological vascular response.

**Poster Number:** E-43
EARLY THREE-DIMENSIONAL ATHEROSCLEROTIC LESION DEVELOPMENT IN SCAVENGER RECEPTOR CLASS B TYPE I-DEFICIENT, HYPOMORPHIC APOLIPOPROTEIN ER61 MICE AND THE ASSOCIATION WITH ENDOTHELIAL AND SMOOTH MUSCLE CELL DAMAGE

L. Zadrozny, B. Lucotte, and R. Balaban
NHLBI, Bethesda, MD

All phases of atherosclerosis have been shown to develop throughout the arterial tree in ApoE-deficient mice, from fatty streaks, to advanced plaques. In the present study, we investigated early lesion development within the descending thoracic aorta in the recently described, SR-BI KO/ApoER61<h>h</h> mouse model of diet-inducible atherosclerosis. To evaluate early lesion development in SR-BI KO/ApoER61<h>h</h> mice, 2-3 month old mice were placed on an atherogenic diet for 21 days. Animals were euthanized daily form 0-5 days and then at 14 and 21 days. Prior to euthanasia, Sytox Blue (SB), a nucleic acid stain that penetrates cells with compromised plasma membranes, was injected into the jugular vein. In-situ imaging of the descending thoracic aorta and intercostal arterial ostia (ICAO) was performed using two-photon excitation fluorescence, sum-frequency generation and Coherent anti-Stokes Raman scattering optical microscopy. Briefly, SB positive smooth muscle cells (SMCs) were consistently located transmurally within the proximal descending aorta, multifocally and circumferentially throughout the ICAO ridges, and interestingly, several pairs of ICAO exhibited a cluster of mural to transmural SB positive SMCs downstream between bilateral pairs of ICAO. Lipid droplets, foam cells and early ICAO plaques often colocalized with SB positive cells in all regions but often spared the vascular bed between bilateral ICAO. SB positive endothelial cells (ECs) were present within the ICAO ridges colocalized with lipid. These findings suggest that EC and SMC damage within these marginal zones downstream to the ICAO may contribute to retention of low-density lipoprotein as propagation of early lesion development prior to fatty streak formation.

CARDIOMYOPATHY IN A CANINE MODEL OF CLN2 DISEASE

G.C. Johnson, S. Leach, B. Williamson, W. Young, L. Castaner, J.R. Coates, B.L. Davidson, and M.L. Katz
The University of Missouri and the Children’s Hospital of Philadelphia Research Institute

A canine model of classical late-infantile neuronol ceroid lipofuscinosis (CLN2 disease) in long-haired Dachshunds results from mutations in the <i>TPP1</i> gene. Dachshunds homozygous for the null mutation have complete deficiency of tripeptidyl peptidase-1, a soluble lysosomal enzyme, with gradual accumulation of storage material in the CNS and other tissues. Affected dogs develop blindness, seizures, cognitive and neurologic deficits beginning at 4 months of age, and are euthanized at average
11 months. At 3 months old, a cohort of 4 affected dogs were injected with recombinant adeno-associated virus expressing TPP1 (rAAV.caTPP1) into the lateral ventricle of the brain. Treated dogs showed delayed onset clinical signs, disease progression and had extended survival up to 21 months of age. During gross necropsy of animals humanely euthanized, the hearts, predominantly the right ventricles, were enlarged and flaccid. Cardiac weight measurements confirmed right ventricular hypertrophy at 10 months of age (2 dogs), and biventricular hypertrophy after 18 months (2 dogs). Histopathology showed foci of cardiomyocyte necrosis and variable interstitial cardiac fibrosis, although tissue staining showed evidence of TPP1 protein in scattered myocytes. Longer lived dogs also had mild increase in pulmonary alveolar macrophages. These findings support that CNS treatment of TPP1-deficient dogs is not sufficient to protect the extraneural tissues such as the heart from disease progression. Findings suggest that treatment needs to be administered both systemically and into the CNS to attenuate disease progression in severe TPP1 deficiency.

**Poster Number:** E-45

**Section:** Experimental Disease  
**Keyword:** Cardiovascular

**A NOVEL WAY OF EPITHELIAL SPREADING: LESSONS FROM THE DEVELOPING EPICARDIUM**

J.G. Vilches-Moure and C.A. Erickson  
University of California-Davis, Davis, CA

The epicardium is derived from the pro-epicardial organ (PEO), which spreads over the developing cardiac tube purportedly as an epithelium and then undergoes an epithelial-to-mesenchymal transition (EMT) to generate vascular endothelium, smooth myocytes, and fibroblasts. Our real-time, in-vitro imaging studies revealed that epicardial cells spread as a loose association of cells and deploy numerous filopodia and lamellipodia, unlike a canonical epithelium, which only generates migratory processes at the leading margin. Analysis of junctional proteins by immunofluorescence reveals that the spreading epicardium has a unique distribution of junctional proteins that differ from either a true epithelium or a true mesenchyme. Taken together, our data show that the spreading avian epicardium has a migratory behavior and phenotype that is intermediate between a true epithelium and a true mesenchyme. Our studies highlight the importance of providing a live-cell context in which to interpret data obtained from fixed tissues, and contribute to the growing evidence that many embryonic tissues represent a continuum between epithelial and mesenchymal tissues. The adult epicardium retains many features of its embryonic precursor. In the adult heart, an epicardial EMT is stimulated in response to pathologic processes such as myocardial ischemia and infarction, and contributes to myocardial fibrosis and remodeling. Thus, a better understanding of the epicardial spreading (and EMT) during normal development may provide insights into this process as it occurs in the adult organism during pathological conditions.

**Poster Number:** E-47

**Section:** Experimental Disease  
**Keyword:** Bone and Joint
EFFECTS OF OXIDIZED LOW DENSITY LIPOPROTEIN ON AN IN VITRO MODEL OF OSTEOARTHRITIS

K. Kuroki, C.R. Kennedy, A.M. Stoker, and J.L. Cook
Comparative Orthopaedic Laboratory, University of Missouri, Columbia, MO

Patients with primary osteoarthritis (OA) commonly have cardiovascular disease (CVD) and it has been reported that cardiovascular mortality is directly proportional to the extent of OA in affected human individuals. It has been hypothesized that oxidized low density lipoprotein (oxLDL), a causative material of atherosclerosis, is a key molecule that connects these diseases. The aim of this study was to test the hypothesis that oxLDL would exacerbate adverse joint health effects induced by IL-1β in an in vitro model of canine osteoarthritis. All procedures were approved by the institution's animal care and use committee. Six mm cartilage and four mm infrapatellar fat pad explants were obtained from 6 dogs that were euthanized for reasons unrelated to the project. Cartilage and fat pad explants were co-cultured in medium with either media alone (Control), recombinant canine IL-1β (2ng/ml), oxLDL (100ug/ml) or IL-1β+oxLDL for 21 days. Samples of liquid media (n=6 of each group) were collected and refreshed every 3 days of culture for biochemical evaluation. Explants (n=6) were collected on day 21 for evaluation of cell viability, glycosaminoglycan content, and histopathology. Although nitric oxide concentrations in the IL-1β+oxLDL was significantly higher than all other groups at respective time point, contradictory to our hypothesis, oxLDL did not exacerbate adverse IL-1β effects as determined by chondrocyte viability, histopathology, concentrations of IL-6, IL-8, MCP-1 and Gro-α and MMP activities. This study implicates a possible and complex role of oxLDL in joint health/pathology.

Poster Number: E-48

Section: Experimental Disease
Keyword: Bone and Joint

EVALUATION OF PARTIAL TRANSECTION VERSUS SYNOVIAL DEBRIDEMENT OF THE ACL AS NOVEL CANINE MODELS FOR MANAGEMENT OF ACL INJURIES

C.C. Bozynski¹, K. Kuroki¹, J.P. Stannard², P.A. Smith², A.M. Stoker¹, C.R. Cook¹, and J.L. Cook¹,²
¹University of Missouri, Comparative Orthopaedic Laboratory and ²Department of Orthopaedic Surgery, ³Columbia Orthopaedic Group, Columbia, MO, USA

Anterior (cranial) cruciate ligament (ACL) tears are common injuries in humans and dogs. Besides the pain and dysfunction associated with ACL tears, ACL deficiency significantly increases the risk of early onset knee osteoarthritis (OA). ACL research is currently hindered by a lack of valid pre-clinical models. Importantly, the ACL is normally extra-synovial, which is critical to ligament and joint health. Although this property of the ACL is lost when its synovial sheath is compromised by injury, it has been given little attention with respect to its roles in post-ACL mechanisms of disease. This study characterizes the effects of partial transection versus synovial debridement of the ACL in dogs. With IACUC approval, twenty-seven adult hounds were randomized into three groups and assessed over 8
weeks: sham control, intact ACL with synovial debridement (exposed ACL), and partial transection of the ACL (partial tear ACL). More severe ACL/whole-joint pathology and radiographic scores for OA were present in the partial tear ACL group when compared to exposed and/or sham control groups, which correlated with more severe clinical assessments. In conclusion, biologic components of ACL injury (i.e., exposed ACL) played a role in whole-joint inflammation, but the clinical and pathological effects were more severe when both biologic and biomechanical components were present (i.e., partial tear ACL). These novel canine models were successfully developed and validated for pre-clinical ACL research and are being used to evaluate treatment options for acute management of ACL injuries.

**Poster Number:** E-49

**Section:** Experimental Disease  
**Keyword:** Respiratory System

**CALPROTECTIN EXPRESSION IS CONSTITUTIVELY ALTERED IN THE CYSTIC FIBROSIS PIG AT BIRTH PRIOR TO THE ONSET OF NEUTROPHIL MEDIATED INFLAMMATION**

D.K. Meyerholz\(^1\), L. Paemka\(^2\), D.A. Stoltz\(^3\), M.J. Welsh\(^3\), P.B. McCray\(^2\), and R.D. Gray\(^4\)

\(^1\)Department of Pathology, University of Iowa, Iowa City, Iowa, USA,  
\(^2\)Department of Pediatrics, University of Iowa, Iowa City, Iowa, USA,  
\(^3\)Department of Internal Medicine, University of Iowa, Iowa City, Iowa, USA,  
\(^4\)School of Clinical Sciences, University of Edinburgh, Edinburgh, Scotland

Calprotectin (S100A8/A9 complex) has emerged as a biomarker in cystic fibrosis (CF) lung disease. The source of calprotectin is often postulated to be neutrophils, but can be measured before the onset of severe inflammation suggesting that increased calprotectin levels in early CF are from another cellular source. We utilized the pig model to investigate calprotectin expression in newborns and compared to human tissue. In newborn wild type (WT) animals S100A8 and S100A9 transcript expression was higher in the distal lung than the large airways. Calprotectin immunostaining was localized at the apical epithelial surface of terminal airways and alveoli in newborn pigs and calprotectin expression was higher in CF than WT pigs. In older CF animals with lung disease, calprotectin staining was associated with leukocytes such as neutrophils and macrophages in CF lung sections. S100A8 and S100A9 expression were higher in cultured primary CF pig airway epithelia than WTs when measured by QrtPCR (p<0.01 and p<0.05 respectively). This finding was replicated in human primary cultures (S100A8 p<0.01, S100A9 p=ns). S100A8 and S100A9 expression in neutrophils from CF pigs trended higher although this failed to reach significance; human neutrophils followed a similar pattern and the increased expression of S100A8 was significant (p<0.05). We also demonstrate that increased calprotectin expression may be a constitutive defect in CF epithelial cells and neutrophils. Taken together these data suggest that absence of CFTR is associated with increased calprotectin expression and that the source of excess calprotectin is the epithelial cell in early disease and the neutrophil in later disease.

**Poster Number:** E-50

**Section:** Experimental Disease  
**Keyword:** Respiratory System
MORPHOLOGIC ARTIFACTS RELATED TO INSUFFLATION MATERIAL FOR LUNG CRYO SECTIONING

Department of Pathology, Roy J. & Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA

Sectioning of frozen tissue is commonly utilized in research pathology for histopathologic diagnosis, morphometry, immunohistochemistry and immunofluorescence. When freezing lung tissue, it can be a challenge to maintain insufflation of pulmonary alveoli while avoiding water crystals formation and other freezing artifacts. Lung insufflation for frozen sectioning has been described using multiple compounds including agarose, saline and embedding media such as OCT (optimum cutting temperature). We sought to determine the optimal alveolar inflation material for frozen sectioning of murine lungs to attain optimum alveolar morphology. Specifically, we evaluated lung insufflation of phosphate-buffered saline (PBS, isotonic) versus various dilutions of OCT with our endpoints being tissue morphology using HE staining. PBS insufflation alone caused severe ice crystal formation and subsequent tissue disruption. Inflation with undiluted OCT preserved the tissue architecture beautifully but caused moderate to sometimes marked dilation of airways with flattening of the airway epithelial cells. This is likely due to the enhanced air trapping that occurred secondary to the viscous and undiluted OCT in airways. Interestingly, the diluted OCT at both 1:1 and 2:3 dilutions with PBS did not cause the airway dilation or epithelial flattening, but did instead caused smudging of tissue architecture, thickening of alveolar septa and swelling of nuclei. We hypothesize that this result is due to lung tissue specific effect and in our future studies we are testing various solutions to define a mechanism and a viable solution.

**Poster Number:** E-51

**Section:** Experimental Disease

**Keyword:** Respiratory System

COMPARISON OF IBA1 LOCALIZATION IN COMMON MODEL SPECIES USED TO STUDY HUMAN LUNG DISEASE

D.K. Meyerholz¹, A.M. Lambertz¹, H.A. Flaherty², K.N. Gibson-Corley¹
¹Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, Iowa, ²Department of Veterinary Pathology, Iowa State University College of Veterinary Medicine, Ames, Iowa

Macrophages of the lung play important roles in health and in disease. We evaluated the macrophage marker IBA1 for its potential use by immunohistochemistry in various species to study human lung diseases. In our pilot study, we evaluated iba1 in lungs from pigs (n=4), ferret (n=2), rat (n=2), sheep (n=2), mice (n=6) as well as humans (n=3). Multifocal IBA1 staining was often seen in the perivascular spaces and alveolar septa. Immunostained cells were also seen in the airway walls subjacent to the surface epithelium and extending at times into it. Alveolar macrophages generally had weak immunostaining in the healthy lung. In contrast, regions with lung inflammation and / or disease had alveolar macrophages with robust IBA1 immunostaining. Interestingly, in species such as the pig with
intravascular macrophages, these too had immunostaining. Lung tissues from other species commonly used for lung research such as mice, rats, ferrets, and sheep were examined and showed similar distribution and staining qualities as in the pig lung. We then asked whether this marker could be applicable for translational studies to human lungs. Cellular immunostaining had a similar qualitative distribution pattern as observed in animal models. Minor variations in staining of alveolar macrophages between and within species are likely attributable to the current health status, environmental exposures, or terminal cases (often seen in autopsy studies). Immunocytochemistry techniques are being evaluated. Our findings suggest IBA1 could be a useful marker of interstitial and activated macrophages in species used for translational lung research.

**Poster Number:** E-52

**Section:** Experimental Disease  
**Keyword:** Nervous System

**A NOVEL PORCINE MODEL OF ATAXIA TELANGIECTASIA REPRODUCES NEUROLOGICAL FEATURES AND MOTOR DEFICITS OF HUMAN DISEASE**


1University of Iowa Carver College of Medicine, Iowa City, IA, 2Sanford Children's Health Research Center, Sanford Research, Sioux Falls, SD, 3Exemplar Genetics, Sioux Center, IA

Ataxia telangiectasia (AT) is a progressive multisystem disorder caused by mutations in the AT-mutated (ATM) gene. AT is a neurodegenerative disease primarily characterized by cerebellar degeneration with loss of Purkinje cells in children leading to motor impairment. The disease progresses with other clinical manifestations including oculocutaneous telangiectasia, immune disorders, increased susceptibility to cancer and respiratory infections. Although genetic investigations and physiological models have established the linkage of ATM with AT onset, the mechanisms linking ATM to neurodegeneration remain undetermined, hindering therapeutic development. The current murine models carrying mutated ATM have shown high fidelity in mimicking the ancillary symptoms of AT. However, the hallmark neuropathological phenotypes of Purkinje cell loss have not been fully recreated to date, thus highlighting the need for a more suitable animal model. Utilizing gene targeting and somatic cell nuclear transfer strategies, we engineered a novel porcine model of AT in order to bridge the gap between patients and mouse models and ultimately, unmask basic mechanisms underlying the neuropathology of the disease. Newborn AT pigs had significant loss of Purkinje cells and altered cytoarchitecture compared to littermate controls suggesting fetal origins for AT lesions. Evaluation of 4 month old pigs showed that AT pigs had several motor deficit phenotypes. The porcine model of AT is the first animal model that faithfully mimics the neuropathological features seen in AT patients. The porcine AT model may become a useful tool to enable pharmacological screening, identify disease mechanisms and potentially, in the future, provide quantifiable endpoints for preclinical therapeutics.

**Poster Number:** E-53

**Section:** Experimental Disease  
**Keyword:** Nervous System
HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES ON THE CANAVAN DISEASE MODEL RAT

M. Kuwamura, S. Tanimura, M. Tanaka, T. Izawa, T. Kuramoto, and J. Yamate
Laboratory of Veterinary Pathology, Osaka Prefecture University, and Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Japan

Animals with spontaneous mutations affecting myelin are useful for studies of mechanisms underlying myelination and myelin maintenance. The tremor (TRM) rat is a spontaneous mutant rat that exhibits spongiform degeneration in the central nervous system. The TRM rat has a genomic deletion involving the aspartoacylase gene, a causative gene for Canavan disease. We conducted detailed histological and ultrastructural studies to elucidate the pathogenesis of the TRM rat.

Immunohistochemical studies revealed that olig2-strongly positive oligodendrocyte precursor cells were decreased in the white matter of the spinal cord in the affected homozygous rats (tm/tm) at 12 weeks of age. In situ hybridization for proteolipid protein (PLP) demonstrated that the number of small, round PLP-positive oligodendrocytes was increased in the white matter of spinal cord in the TRM rats. Ultrastructurally homozygous rats have axonal swelling with filamentous accumulation and myelin splitting in the white matter of the spinal cord.

These findings suggested that TRM rat involves in the impairments of axonal transport and of maturation in the oligodendrocyte lineage.

Poster Number: E-54

Section: Experimental Disease
Keyword: Nervous System

PATHOGENESIS OF FOREBRAIN ABNORMALITIES IN A TERATOGEN-INDUCED MODEL OF OROFACIAL CLEFTING

L. Ansen-Wilson, J. Everson, G. Heyne, and R. Lipinski
University of Wisconsin, School of Veterinary Medicine, Madison, WI

Orofacial clefts (OFCs), including clefts of the upper lip and/or palate, are among the most common birth defects in humans and veterinary species. These conditions are etiologically complex and most cases are believed to result from intricate interactions between genetic and environmental influences. They require extensive medical intervention and can be emotionally and financially devastating for individuals, families, and healthcare providers. Additionally, behavioral and cognitive deficits have been extensively reported in patients with non-syndromic OFCs. These traits were traditionally considered secondary to repeated anesthetic events during childhood, social stigma, and/or speech and hearing complications associated with abnormal craniofacial anatomy. However, increasing evidence supports the alternative hypothesis that the reported deficits are primary manifestations of abnormal brain development. Our recent work using high resolution magnetic resonance microscopy to examine a murine OFC model confirmed that structural brain malformations are congenital, not acquired.
However, coincident molecular alterations have yet to be fully explored and the functional ramifications of these changes remain poorly understood. To further investigate the link between OFCs and neurocognitive deficits, we examined forebrain patterning and development in embryonic mice with teratogen-induced facial malformations. Nuanced disruptions in forebrain specification and a significant deficiency of the medial ganglionic eminence, an important neural progenitor zone, were observed as early as gestational day 11. These findings suggest a novel molecular mechanism for the pathogenesis of behavioral and cognitive deficits that co-occur with OFCs and may serve to shift current understanding and treatment of these conditions.

§ Poster Number: E-55

Section: Experimental Disease
Keyword: Nervous System

**IN SITU IDENTIFICATION OF BRAIN LIPID ALTERATIONS BY IMAGING MASS SPECTROMETRY (IMS) IN MOUSE MODELS OF TOXIN-INDUCED EXPERIMENTAL DEMYELINATION**

R.J. Maganti¹,², X. Hronowski¹, P. Juhasz¹, B. Wipke¹, A.B. Rogers² and R.W. Dunstan¹,²
¹Biogen, Cambridge, MA, ²Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA

Lipids are the most abundant organic molecules in the brain and 60% of these lipids are associated with myelin. Thus neuropathology requires understanding of lipids and their structural relationship to disease. Imaging Mass Spectrometry (IMS) is the only technology that enables in situ identification of simple to complex lipids while providing morphologic context. Herein we applied an improved IMS method to evaluate the signature changes in brain lipid subtypes in mouse models of toxin-induced reversible demyelination.

**Objectives:**
- To define the spatial and temporal changes in mouse brain lipid composition upon cuprizone- and lysolecithin-induced reversible demyelination.
- To correlate lipid changes with the histological changes analysed by whole slide imaging and quantitative immunohistochemistry.

**Methods:**
Brain tissue is harvested from chow-fed, cuprizone-fed and lysolecithin-injected age-matched mice at predetermined study time points, prepared for lipid-IMS and immunohistochemistry with established neural cell markers.

**Results:**
- Demyelination is associated with decreased amounts of sulfatides (major myelin lipids) containing long-chain fatty acids and increased amounts of sulfatides containing short-chain fatty acids.
- These changes correlate with the altered morphology and reverse when the myelin loss is restored.
Conclusions:

- IMS can define previously unrecognized, morphologically-relevant, demyelination-associated patterns of brain lipids.
- The changes in sulfatides implicate the role of lipid-metabolizing enzymes acting in a fatty acyl chain length dependent manner.
- IMS enables correlation of the molecular expression of lipids with morphology in toxin-induced demyelinating diseases. This method should have broad application to understand the role lipids play in other neurologic diseases as well.

Poster Number: E-56

Section: Experimental Disease
Keyword: Nervous System

PROBING THE ROLE OF LOW-THRESHOLD MECHANORECEPTORS IN THE PATHOPHYSIOLOGY OF PAIN

L.K. Crawford¹, X. Dong¹, D.D. Ginty², and M.J. Caterina¹
¹Johns Hopkins University, Baltimore, MD, ²Harvard Medical School, Boston, MA

Neuropathic pain affects a wide variety of patients, and yet, due to our incomplete understanding of the pathophysiology, many current therapies are ineffective. Neurons known as low-threshold mechanoreceptors (LTMRs) may play a significant yet poorly understood role in neuropathic pain, particularly in painful responsiveness to light touch stimuli, termed mechanical allodynia. Discovery of molecular markers that distinguish several LTMR subtypes has enabled characterization of intricate patterns of LTMR innervation in hairy and glabrous skin. Identification of these markers also provides a means for the labeling and targeted manipulation of LTMRs that has been lacking in models of neuropathic pain. We hypothesize that rapidly adapting ABeta LTMRs contribute to neuropathic mechanical allodynia and that dysregulated ABeta LTMRs provide an ongoing source of peripheral activity that drives this pathology. Expression of an inhibitory construct (DREADD) was restricted to sensory neurons using the pan-sensory marker Advillin. Activation of the DREADD in healthy mice reversible decreased mechanical sensation and locomotor activity (presumably due to inhibition of proprioception), with a trend towards decreased thermal sensation. We are currently using genetic labeling of rapidly adapting ABeta LTMRs to elucidate changes in dermal innervation in a mouse model of neuropathic pain. Future studies will use Advillin, LTMR markers, and the DREADD to determine the effects of targeted inhibition of rapidly adapting ABeta LTMRs in the face of neuropathic pain. These data will provide anatomic and functional insight into the role that mechanoreceptors play in pain with implications for toxic neuropathies and a range of systemic diseases.

Poster Number: E-57

Section: Experimental Disease
Keyword: Reproductive System
INVESTIGATION OF CLOMIPHENE CITRATE TREATMENT ON SPERMATOGENESIS AND TESTICULAR HISTOMORPHOLOGY IN RATS

K.N. Gibson-Corley¹, P. Kogan², P.W. Naumann¹, J.A. Goeken¹, and M. Wald²
¹Department of Pathology, ²Department of Urology, Roy J. & Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA

Clomiphene citrate (CC) is used off-label as a treatment for selected cases of male infertility, acting as a competitive selective-estrogen receptor modulator. Although CC is used clinically, there have only been a small number of randomized controlled human studies that have yielded inconclusive findings. Here we have used a prospective animal model for studying CC treatment to determine if this drug increases spermagenesis for treatment of male infertility. We treated sexually mature Sprague-Dawley rats with CC via oral gavage for either 1 or 2 rat spermatogenic cycles (13 days for 1 cycle or 26 days for 2 cycles) at a dose of 0.6 mg/kg. This dose was chosen as it is a similar to dose to what is clinically used in humans. Sperm counts were measured and testes were fixed in Bouin’s and stained with HE for histopathological and morphometric analysis. No histomorphologic differences were noted in the testes treated with CC. Seminiferous tubule minimum diameter and tubular epithelial height were measured and number of retained spermatids was scored and there were no significant differences compared to control. Although previous studies have shown that rats treated with much higher doses of CC (>20 mg/kg) have decreased spermagenesis, we can conclude that CC treatment at 0.6 mg/kg in rats does not have an effect on spermagenesis.

Poster Number: E-59

Section: Experimental Disease
Keyword: Liver and Pancreas

DIETARY IRON OVERLOAD ALTERS HEPATIC INFLAMMATION IN A RAT MODEL OF NONALCOHOLIC STEATOHEPATITIS

T. Izawa, M. Atarashi, R. Miyagi, M. Kuwamura, and J. Yamate
Veterinary Pathology, Osaka Prefecture University, Osaka, Japan

Iron overload is considered as a risk factor for chronic liver diseases, and its role in nonalcoholic steatohepatitis (NASH) is still undetermined. We here investigated the effect of iron overload on NASH pathology in rats. 6 weeks-old male F344 rats were fed control (5% fat, 0.03% Fe), high fat (HF; 16% fat, 0.02% Fe), high iron (HI; 4% fat, 0.5% Fe) and high-fat-high iron diet (HFHI; 16% fat, 0.5% Fe) for 30 weeks. Liver and serum were collected for blood biochemical, histopathologic and molecular biological analyses. Serum and liver iron were increased in HI and HFHI rats. Serum ALT and AST were elevated in HF and HFHI rats; ALT tended to increase in HFHI compared to HF rats. The number of lobular inflammatory foci increased in HF and HFHI rats, with higher number in HFHI than in HF rats. Iron deposits were intense in macrophages within the inflammatory foci. Expression of TNFα, IL1β and TGFβ were higher in HFHI than in HF rats. Cytoplasmic release of apoptosis inducible factor was increased in HF and HFHI rats, with no difference between HF and HFHI. Our results suggested that hepatic iron overload can increase hepatic inflammation, thus exacerbate NASH pathology.
Natural Disease Focused Scientific Poster Presentations

Poster Number: N-1

Section: Natural Disease
Keyword: Liver and Pancreas

LECTIN HISTOCHEMISTRY EVALUATION OF LIVER AND MESENTERIC LYMPH NODE OF BUFFALOES KEPT IN BRACHIARIA SPP. PASTURES

Federal Rural University of Rio de Janeiro, Brazil

Animals grazing Brachiaria spp. commonly present foamy macrophages (FM) isolated or grouped in the liver and crystals within biliary ducts, that are liable for damage the liver leading to accumulation of phylloerythrin. The pathogenesis of formation and the type of material stored in FM is still unknown. This study aims to develop and standardize the use of lectin histochemistry to detect steroidal saponins in fragments of liver and mesenteric lymph node of buffaloes kept in different Brachiaria spp. pastures in Brazil. Tissue from 40 animals were analyzed: 10 buffaloes that were kept in predominant pasture of B. decumbens for 10 months; 10 buffaloes that were kept in pasture with a predominance of B. brizantha for 18 months; 10 buffaloes that were raised on a property that used only B. brizantha to feed; and 10 buffaloes that were maintained in native pasture without Brachiariaspp., as a negative control. Fourteen lectins were evaluated (Con-A, SBA, WGA, DBA, UEA, RCA, PNA, GSL-I, PSA, LCA, PHA-E, PHA-L, SJA and SWGA). Previous studies demonstrated that PNA showed great binding reactivity for FM in bovine and ovine. In this study, it was found that SWGA showed high specificity and great binding reactivity for FM. WGA showed great binding reactivity for FM and also slight binding reactivity for hepatocytes. GSL, PHA-E and PHA-L showed great reactivity but low specificity for FM. Lectin histochemical evaluation can be used to characterize the stored glycoconjugates in FM and provide clues to clarify the pathogenesis of these cells.

Poster Number: N-2

Section: Natural Disease
Keyword: Nervous System

A RETROSPECTIVE STUDY OF CANINE SKULL BASE NEOPLASIA

D.R. Rissi
Department of Pathology, College of Veterinary Medicine, the University of Georgia, Athens, GA

This study describes the prevalence and distribution of 42 cases of skull base neoplasms in dogs between 2000 and 2014. The average age of affected individuals was 9.5 years and there was no sex or breed predisposition. The most common skull base neoplasms were meningioma (25 cases) and pituitary adenoma (9 cases). Less common tumors included craniopharyngioma (2 cases), nerve sheath tumor (2
cases), pituitary carcinoma (1 case), meningeal oligodendrogliomatosis (1 case), presumed nasal or sinonasal carcinoma (1 case), and multilobular tumor of bone (1 case). All tumors caused some degree of compression of adjacent structures. The distribution of the lesions was greatest in the sellar region (18 neoplasms), followed by the paranasal region (12 neoplasms), caudal cranial fossa (10 neoplasms), central cranial fossa (1 neoplasm), and rostral cranial fossa (1 neoplasm). Although a number of reports describing primary and secondary intracranial neoplasia in dogs have been reported in the veterinary medical literature, no work has been conducted to address skull base neoplasia as a distinct entity.

Poster Number: N-3

Section: Natural Disease
Keyword: Nervous System

A NOVEL INHERITED CEREBELLAR ABIOTROPHY IN A COHORT OF RELATED GOATS

J.W. Koehler
Auburn University, Auburn, AL

Cerebellar abiotrophies, also known as cerebellar ataxias, are characterized by premature postnatal degeneration of cerebellar neurons. This report describes the clinical, magnetic resonance imaging (MRI), gross, histopathological, and immunohistochemical features of a novel inherited cerebellar abiotrophy in a cohort of three closely-related mixed-breed goats (Capra aegagrus hircus) in the southeastern United States. The animals all presented with juvenile-onset ataxia, hypermetria, wide-based stance, head tremors, and nystagmus. On MRI and at gross examination, there was moderate thinning of the cerebellar vermis and sharpening of the folia. Histologically, the vermis, paravermis, and flocculonodular lobe had moderate to severe segmental loss of Purkinje cells with sparing of the hemispheres and secondary loss of granule cells and astrogliosis. Heritable cerebellar ataxias have been reported in many domestic animal species but have not, to the authors’ knowledge, been previously reported as a heritable condition in goats.

Poster Number: N-4

Section: Natural Disease
Keyword: Mammary

STROMAL CHANGES WITHIN IN SITU CARCINOMA AND EARLY INVASIVE CARCINOMAS IN MAMMARY GLAND OF FEMALE DOGS WITHOUT CLINICAL SIGNS OF MAMMARY TUMOR: HISTOPATHOLOGIC STUDY

J. Caicedo, N. Martinez, and C. Iregui

Background: During the development of mammary gland and cancer progression, the stroma is dynamically altered in composition, amount and orientation. Through various molecular mechanisms, these stromal alterations can exert different effects on the epithelial cells and on occasion can favor
their migration through the basement membrane (BM) and invasion of the interstitium and blood vessels. **Objective and Methods:** The aim of this study was to characterize histologically the morphology of the stroma in 12 mammary glands containing 42 carcinomas in situ (CaIS) and 7 early invasive carcinomas (Cal) from 6 female dogs without clinical signs of tumor. The parameters of evaluation included: BM by Periodic Acid Schiff (PAS), stromal density by Trichrome Masson, and inflammatory cell type by regular H&E. **Results:** The BM was thickened in CaIS and interrupted in Cal. The connective tissue was dense in both types of lesions (CaIS and Cal). Infiltration of lymphocytes, plasma cells and macrophages were predominant in both lesions. A positive correlation between high nuclear grade of CaIS and thickness of the BM (p<0.0001) and also between high nuclear grade of CaIS and the increase of connective tissue surrounding altered lobules or ducts was observed (p<0.0085). A positive correlation was also noted between BM thickness and connective tissue density located nearby these altered lobules with high nuclear grade CaIS (p<0.0706). **Conclusion:** Our findings suggest that the BM and stroma interact during progression of CaIS and Cal in the altered lobes of MG in female dogs and are important components of these lesions.

**Poster Number:** N-5

**Section:** Natural Disease  
**Keyword:** Wildlife

**ENDOGENOUS LIPID PNEUMONIA IN A 15 YEAR OLD AFRICAN LYNX (CARACAL CARACAL)**

R.M. Tan¹ and R. Dhuët²  
¹IDEXX Reference Laboratories, Inc., West Sacramento, CA, ²All Creatures Animal Hospital, New Iberia, LA

A 15-year-old, male African lynx was presented for a history of chronic, increased expiratory effort that was unresponsive to antibiotics and corticosteroids. The lynx was sedated for blood collection and radiograph purposes. Clinical pathologic testing revealed an incidental hyperglycemia and glycosuria, indicating diabetes mellitus. Generalized mild opacification was noted in the lung using X-ray. The lynx expired as it was about to be transported home, despite adequate recovery procedures. At necropsy, several 0.5-1 mm white plaques were noted on the pleural and cut surfaces of the lung. Only the lungs were submitted for histologic evaluation. Histopathology revealed alveolar aggregates of foamy macrophages, multinucleated giant cells and cholesterol clefts, consistent with endogenous lipid pneumonia. Using Oil Red O stain, the nature of the vacuoles was determined to be lipid. To the best of the authors’ knowledge, this is the first reported case of endogenous lipid pneumonia in an African lynx. As with most previous reports of endogenous lipid pneumonia in other domestic and wildlife species, the authors conclude that this is an incidental finding of no clinical significance to the cause of death of the animal, which remained undetermined.

**Poster Number:** N-6

**Section:** Natural Disease  
**Keyword:** Nervous System
BETA-AMYLOID OLIGOMER, NEUROFIBRILLARY TANGLES AND NEURONAL LOSS IN BRAINS OF AGED DOMESTIC CATS


Although many animal species develop senile plaques (SPs) composed of beta-amyloid (Aβ) protein, neurofibrillary tangles (NFTs) and subsequent neurodegeneration, which are the integral part of Alzheimer’s disease (AD) pathology is rarely observed in animals. We have found Aβ deposition and NFTs frequently in aged Felinae species including the domestic cat. NFTs were composed of hyperphosphorylated tau protein, and were located in the entorhinal cortex, hippocampus and temporal cortex, comparable to AD patients. Interestingly, these felids did not develop argyrophilic SPs, instead small aggregates of Aβ were observed. The Felinae species express Aβ with a different amino acid sequence compared to that of human and other animals displaying SPs. We further studied the propensities of Aβ and tau proteins extracted from brains of domestic cats by immunohistochemistry, Western blot and dot blot analyses. Aβ oligomers were detected in the cytoplasm of hippocampal pyramidal cells as the predominant Aβ species and were vulnerable to formic acid. Like in human, both 3-repeat and 4-repeat tau isoforms were expressed also in adult cats. Moreover, NFTs were composed of both tau isoforms. In cats with severe NFTs, the number of hippocampal neurons was significantly decreased compared to age-matched controls. Accumulation of Aβ oligomers is likely to be related to the high occurrence of NFTs in aged cats. In conclusion, the cat exhibits, Aβ oligomers, NFTs and neuronal loss that are involved in the pathological cascade of AD.

Poster Number: N-7

Section: Natural Disease
Keyword: Infectious Disease

PHAEOHYPHOMYCOSIS OF THE UPPER RESPIRATORY TRACT IN FOUR HORSES

J.B. Harvey1, L.B. Mamo2, L.B. Borst1, T. Prange2, P. Bzikova3, and K.E. Linder1
1Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, 2Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Upper respiratory tract phaeohyphomycosis is relatively common in the horse; however, reports of phaeohyphomycosis are lacking. In this retrospective study, upper respiratory tract phaeohyphomycosis is described in four, 10 to 14 years-old, castrated male horses of different breeds. Presenting problems were respiratory noise and nasal masses in two horses, epistaxis in one horse, and dropping food, halitosis, and nasal discharge in one horse. Clinically, masses were identified on the nasal septum of two horses, in the guttural pouch of one horse and in the rostral maxillary sinus of one horse. Histologically, neutrophilic to pyogranulomatous inflammation contained pigmented fungi that varied from spherical, yeast-like forms to elongated, 5-10 um wide, septate hyphae, some with bulbous ends. Eosinophils were rare. Fungal cultures from two horses grew Exophiala sp. (one horse) and both Cladosporium sphaerospermum and Curvularia sp. (one horse). PCR for ITS2 identified fungal DNA in 3/5 paraffin
biopsy samples from four horses but a 98% or greater identity match occurred in only one horse, consistent with *Curvularia sp.*, which matched a culture result. Treatments included systemic antifungals, surgical debulking, or a combination of both, and resulted in resolution of clinical signs (one horse) or clinical improvement (two horses) with recurrent episodes over 5 months or 2 years. One horse was lost to follow-up. Equine phaeohyphomycosis of the upper respiratory tract causes pyogranulomatous masses in variable anatomical locations (nasal, guttural pouch, and sinus) and responds variably to antifungal treatment and/or surgical debulking, persisting in one horse for 2 years post initial presentation.

**Poster Number**: N-8

**Section**: Natural Disease  
**Keyword**: Respiratory

**ENVIRONMENTAL TOXICANTS AND INNATE PULMONARY DEFENCE: THE EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS ON IN VITRO EXPRESSION OF BETA DEFENSINS IN TRACHEAL EPITHELIAL CELLS**


Bacterial pneumonia is an important cause of morbidity and mortality in cetaceans, including populations experiencing unusual mortality associated with environmental contamination by crude oil and polyaromatic hydrocarbons (PAHs). These findings may suggest toxicant-induced suppression of respiratory defences as a mechanism by which oil spills compromise the health of cetaceans. PAHs are constituents of fossil fuels and aerosolized by-products of combusive processes. Aerosolized or aspirated PAHs may be metabolized by airway epithelium and are known to interact with the aryl hydrocarbon receptor which can affect NF-kB signalling. Since inducible expression of b-defensin antimicrobial peptides is known to be dependent on NF-kB, we hypothesized that PAHs suppress b-defensin expression in tracheal epithelial cells. Initially, we modeled this response by measuring the effects of specific PAHs (phenanthrene and benzo(a)pyrene) on the inducible expression of the b-defensin tracheal antimicrobial peptide in bovine tracheal epithelial cells. Primary cultures of bovine tracheal epithelial cells exposed to 0.158 or 1.58 μM of phenanthrene for 48 hours, with or without 16 hours of stimulation with lipopolysaccharide, were analyzed for tracheal antimicrobial peptide gene expression by quantitative RT-PCR. Exposure to phenanthrene significantly (*P*=0.0016) suppressed lipopolysaccharide-induced gene expression of tracheal antimicrobial peptide to 70% of baseline. Further experiments are ongoing with other PAHs including benzo(a)pyrene, as well as crude oil extracts. These are the first data to show that exposure to PAHs suppresses b-defensin gene expression, suggesting a mechanism by which environmental contaminants in oil spills suppress innate respiratory defenses and thereby lead to pulmonary disease.

**Poster Number**: N-9

**Section**: Natural Disease  
**Keyword**: Wildlife
INTESTINAL PARASITISM IN THREE WILD HOWLER MONKEYS

A. Berrocal, J. Hernández, and K. Carvajal

The howler monkeys (*Alouatta palliata*) are found throughout Central America. In Costa Rica it is present in both dry and rainforest. There are few studies reporting the presence of intestinal parasites in this species all done on fecal samples. To our knowledge this is the first pathological and parasitological report of intestinal damage caused by nematodes infestation in this species.

Cases: A. An adult female and two adults males (B and C) wild howler monkeys from the Pacific Coast were found dead and a complete necropsy was performed. Cases A and B, a cecum impaction with large numbers of nematodes was observed. In monkey C, parasites were not identified. In all cases multiple samples from the cecum and colon were taken for histopathological examination. Additionally, cecal and cecum feces and adult specimens were submitted for parasite identification.

Histopathology: In monkeys A and B, mucosal necrosis with invasion of nematodes was observed. Besides, in all three monkeys, there was an infiltration of lymphocytes, histiocytes and few eosinophils. Additionally, monkey C it showed hyperplasia of associated lymphoid tissue.

Parasitology: *Enterobius vermiculatus*, was reported in monkey A and *Trypanoxyuris sp* in B. Eggs of Controrchis sp, Strongyloides and Trypanoxyuris sp were found in monkey C.

Despite the fact that the parasitic species found in this paper have already been reported in the literature no one had described the intestinal histopathological changes associated with the infestation. In addition, these monkeys inhabit forest touristic areas and could pose a zoonotic threat to human visitors.

**Poster Number:** N-10

**Section:** Natural Disease

**Keyword:** Endocrine

WILDLIFE SEQUENCES OF ISLET AMYLOID POLYPEPTIDE (IAPP) IDENTIFY CRITICAL SPECIES VARIANTS FOR FIBRILLIZATION

J.S. Fortin and M-O Benoit-Biancamano
Département de Pathologie et de Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada

Amyloid can be detected in the islets of Langerhans in a majority of type 2 diabetic patients. These deposits have been associated with β-cell death, thereby furthering diabetes progression. IAPP amyloidogenicity is quite variable among animal species, and studying this variability could further our understanding of the mechanisms involved in the aggregation process. Thus, the general aim of this study was to identify IAPP isoforms in different animal species and characterize their propensity to form fibrillar aggregates. In this study, we examined the sequence of 38 different wildlife animals. The IAPP gene was isolated and the 37 amino acids of the IAPP peptide were covered for the sequencing. A library of 23 peptides was designed to study the amyloid formation. The amyloidogenic potential of each IAPP homolog was assessed *in vitro* using physicochemical analyses including thioflavin-T assays (ThT), Congo red binding assays, circular dichroism (CD) spectrometry, transmission electron microscopy (TEM) and cell viability assays in pancreatic INS-1 cells. Amyloid formation was impeded when the NFLVH motif
found in segment 8-20 was substituted by DFLGR or KFLIR motif. A 29P, 14K and 18R substitution were often present in non-amyloidogenic sequences. Non-amyloidogenic sequences were obtained from the golden lion tamarin (Leontopithecus rosalia), the common bottlenose dolphin (Tursiops truncates) and the alpaca (Vicugna pacos). To conclude, this project advances our knowledge on the comparative pathogenesis of amyloidosis in type II diabetes. It is conceivable that the additional information gained may help point towards new therapeutic strategies for patients with type 2 diabetes.

**Poster Number:** N-11

**Section:** Natural Disease  
**Keyword:** Neoplasia

**NAPSNIN-A AS AN IMMUNOHISTOCHEMICAL MARKER OF THYROID DIFFERENTIATION IN CANINE THYROID NEOPLASIA**

J.A. Ramos-Vara, C.B. Frank, D.M. DuSold, and M.A. Miller

Three immunohistochemical markers commonly used to document thyroid origin of neoplasms are thyroglobulin, calcitonin, and thyroid transcription factor-1 (TTF1). Pax-8 has been proposed as an additional marker in canine thyroid neoplasia. Napsin-A, an aspartic proteinase involved in surfactant production, labels human pulmonary carcinomas and has been proposed as a marker of canine pulmonary carcinomas. We examined 108 formalin-fixed, paraffin-embedded thyroid tumors (2 carcinomas, 77 follicular carcinomas, 1 medullary hyperplasia, 1 medullary adenoma, 22 medullary carcinomas, 5 mixed follicular-medullary carcinomas) and 6 metastases with a rabbit monoclonal antibody to napsin-A (clone ACI 3043, Biocare, Concord, CA) and compared results with a mouse monoclonal antibody to Pax-8 (clone BC12, Biocare). Antigen retrieval used citrate buffer in a decloaker chamber. Immunoreactivity was scored by the percent neoplastic cells with cytoplasmic (Napsin A) or nuclear (Pax-8) reactivity as 0 (<1%), 1 (1-15%), 2 (16-50%), or 3 (>50%). Ninety (84%) tumors were positive for Napsin A; ninety-two (85%) tumors were positive for Pax-8. Only one case (0.9%) was double-negative. All medullary tumors and five mixed follicular-medullary carcinomas expressed Napsin A. Sixty-five (79%) and 79 (96%) of 82 follicular and mixed follicular-medullary neoplasms expressed Napsin A and Pax8, respectively. One metastatic medullary carcinoma expressed both markers. The other 5 metastases corresponded to follicular carcinomas; only 1 expressed Napsin A, whereas all 5 expressed Pax8. In summary, Pax8 is more sensitive than Napsin A for canine thyroid neoplasms, particularly their metastases. However, the combined use of both markers increased the immunohistochemical sensitivity to 99%.

**Poster Number:** N-12

**Section:** Natural Disease  
**Keyword:** Alimentary

**ALTERATIONS IN THE EQUINE FECAL MICROBIOME ASSOCIATED WITH ANTIMICROBIALS DURING HOSPITALIZATION FOR ELECTIVE SURGICAL PROCEDURES**
J.B. Engiles, D. Pitta, N. Indugu, D. Stefanowski, B. Vecchiarelli, S. Stewart, and L. Southwood

Because alterations in colonic microbiota can produce devastating outcomes for equine patients, an observational pilot study was performed to evaluate changes in the equine fecal microbiome associated with peri-operative antimicrobials administered during hospitalization for elective surgical procedures. Thirty horses (12 Thoroughbreds, 8 Warmbloods, 6 Standardbreds, and 4 crossbred/other breed) without history of antimicrobial administration were admitted for elective musculoskeletal, upper respiratory or other soft tissue surgical procedure. Average duration of hospital stay was 5.5 days (range 3-14). Duration of peri-operative antimicrobials varied from ≤36 hours to ≥48 hours. Horses received intramuscular procaine penicillin G, a combination of intravenous gentamicin and potassium penicillin, or no peri-operative antimicrobials. Pre- and post-operative fecal samples were obtained from the stall floor at regular intervals until hospital discharge. Bacterial 16S rDNA was amplified from genomic DNA extracted from 1g frozen feces using V1V2 primers. 16S pyrosequence reads were analyzed using the QIIME 1.8 pipeline, followed by taxonomic assignments and analyses of community similarity (β-diversity) to generate principal coordinate analyses (PCoAs). Non-parametric permutational multivariate ANOVA tests evaluated the effects of study group categories on overall community composition. Significant differences in microbial community composition were identified between horses undergoing elective surgical procedures treated with peri-operative antimicrobial drugs, compared to those that were not. This study shows that antimicrobial treatment in equine patients undergoing elective surgery impacts the equine fecal microbiome. Future studies will be directed toward evaluating the impact of intestinal microbiome changes on clinical intestinal disease and histologic lesions, and the effect of probiotics.

Poster Number: N-13

Section: Natural Disease

Keyword: Bone and Joint

EQUINE SILICATE ASSOCIATED OSTEOPOROSIS MIMICKING OSTEOLYTIC NEOPLASIA

R. Zavodovskaya, M. Eckert, B. Murphy, S.M. Stover, and S. Diab

An adult paint horse gelding from the central coast of California was presented for evaluation of back pain and left thoracic limb lameness. Radiographs revealed widespread, discrete, radiolucent skeletal lesions and diffuse bronchointerstitial pulmonary infiltrates. Other routine clinical diagnostic results were unremarkable. Euthanasia was elected because of the concern about quality of life. Post mortem examinations revealed widespread, discrete, osteolysis with numerous, large osteoclasts indiscriminately resorbing cortical and trabecular bone in the axial, but also the appendicular skeleton. Thoracic lesions were characterized by miliary, fibrosing granulomatous pneumonia and thoracic lymphadenitis with intralesional necrosis and crystal deposition. The radiographic ante mortem lesions were consistent with osteolytic neoplasia. However, the microscopic characteristics of skeletal lesions coupled with thoracic disease were diagnostic for silicate associated osteoporosis (SAO). This case provides a novel differential for discrete, widespread osteolysis and demonstrates a new lesion pattern for this enigmatic equine disease from California.
DIAGNOSTIC FEATURES OF CANINE SPINAL CORD GLIOMAS

A.L. Burnum¹, A.D. Miller², and D.R. Rissi¹
¹Department of Pathology and Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA, ²Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University, Ithaca, NY

Spinal cord gliomas are uncommonly reported in dogs. This study describes the clinicopathologic and diagnostic features of six cases of canine spinal cord gliomas. Five females and one male were affected with a median age at presentation of 7 years. Four of the six dogs had a brachycephalic conformation (two Boxers, one Boston terrier, and one French bulldog). Clinical course lasted from 3 days to 3 months, and signs ranged from unilateral hind limb lameness to paraplegia. Some dogs had pain upon back manipulation. Clinical signs were rapidly progressive until euthanasia in all cases. All dogs had a presumptive clinical diagnosis of a spinal cord tumor based on the presence of focally extensive intraparenchymal hyperintensity on magnetic resonance imaging (MRI) or enlargement of the cord on computerized tomography (CT) scan. Tumor localization during necropsy and histopathology confirmed MRI and CT findings and ranged from C1 to L6, with no clear segmental predilection. The diagnosis was based on histology and immunohistochemistry in all cases, consisting of four oligodendrogliomas, one astrocytoma, and one gliomatosis cerebri. Oligodendrogliomas were consistently immunoreactive for Olig2 and negative for glial fibrillary acidic protein (GFAP). One case was immunoreactive for synaptophysin. GFAP immunoreactivity was present in the astrocytoma and gliomatosis cerebri case. The latter was also CD18-positive. Neuron-specific enolase was negative in all cases. These results further clarify the incidence of canine spinal gliomas and highlight the need for comprehensive immunohistochemistry in diagnosing these tumors.

AUTOPHAGY IN CANINE APPENDICULAR OSTEOSARCOMA: EXPRESSION PATTERNS OF POSITIVE AND NEGATIVE REGULATORS

C.R. Schott and G.A. Wood
Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON

One year survival in canine appendicular osteosarcoma is improved by the addition of adjuvant chemotherapy, but long term survival is still poor with most of these dogs succumbing to pulmonary metastasis. The process of autophagy has the potential to enhance survival of neoplastic cells following exposure to chemotherapy and may play a role in resistance of osteosarcoma to conventional
treatment. To investigate the expression of autophagy markers in canine appendicular osteosarcoma immunohistochemistry was performed on a small tissue microarray containing samples from a population of dogs who began standard of care treatment for appendicular osteosarcoma (n=20), as well as samples from other common canine tumours. Both negative and positive regulators of autophagy were examined: phospho-mTOR, phospho-AKT1, phospho-S6 ribosomal protein (S6RP), Beclin-1, LC3, and p62. In osteosarcoma, varying intensities of cytoplasmic labeling were observed for phospho-S6RP, LC3, and Beclin-1—a protein required for the initiation of autophagy. Strong Beclin-1 positivity was only observed in cases with <1 year survival (5/13) but was never observed in cases with >1 year survival (0/7). The most intense phospho-S6RP labeling was identified in regions of osteoid/chondroid matrix production, with poor labeling of fibroblastic tumours. p62 and phospho-AKT1 labeling varied in both the nuclear and cytoplasmic compartments. Phospho-mTOR labeling was almost exclusively nuclear, with the exception of cells undergoing mitosis which often displayed strong cytoplasmic positivity. Based on these preliminary findings, the expression of autophagy markers will be examined on a large tissue microarray where it will be possible to identify statistically significant correlations with outcome.

Poster Number: N-16

Section: Natural Disease

Keyword: Wildlife

PATHOLOGY AND CAUSES OF DEATH OF STRANDED CETACEANS IN THE CANARY ISLANDS (2006-2012)

J. Díaz-Delgado¹, M. Arbelo³, E. Sierra¹, S. Sacchini¹, D. Zucca³, J. de la Fuente³, N. García-Álvarez¹, Y. de Quirós¹, M. Andrada¹, O. Quesada-Canales¹, Y. Paz¹, A. Suarez-Bonnet¹, A. Vela³, L. Dominguez², M. Dominguez³, and A. Fernandez¹

¹Veterinary Histology and Pathology, Institute of Animal Health, Veterinary College, University of Las Palmas de Gran Canaria, Trasmontana s/n, Arucas 35413, Las Palmas de Gran Canaria, Spain,
²Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Avenida Puerta de Hierro s/n, 28040-Madrid, Spain, ³Servicio de Inmunología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

This study describes the epidemiology, pathological findings and probable causes of death (CD) (‘pathological entities’) of 236/320 (73.75%) stranded cetaceans representing 3 and 17 species of mystectes and odontocetes, respectively. From 236, 111 (47.03%) were females, 115 (48.72%) males. Fifty-two (22.03%) were neonates/calves, 65 (27.54%) juveniles/subadults, 118 (50%) adults. Sixty-six (27.96%) were in good nutritional status (NS), 60 (25.42%) moderate, 64 (27.11%) poor, 16 (6.78%) emaciated. A CD was recognized in 220/236 (93.22%). Within natural pathological categories, those associated with good NS involved 81/220 (36.81%) from which 11 died of brevetoxicosis; whereas 49/220 (22.27%) presented significant loss of NS. Fatal intra- interspecific traumatic interactions included 37/220 (16.81%). Ship collisions were determined in 24/220 (10.90%). Neonatal/perinatal pathology enrolled 14/220 (6.36%). Interaction with fishing activities encompassed 10/220 (4.54%). Foreign body pathology was observed in 5/220 (2.27%). Within natural categories CD were infectious (58%) with confirmed known pathogens (morbillivirus, herpesvirus, Erysipelothrix rhusiopathiae, Photobacterium damselae, Aspergillus fumigatus) and some newly recognized (Streptococcus
phocae, Wolfhartiimonas chitiniclastica, Mycoplasmasubsp. phocaen). Parasitic disease represented 25% with fatal cases involving Toxoplasma gondii, Nasitrema sp., Crassicauda sp., Brachycladium atlanticum, Oschmarinella rochebruni, Pholeter gastrophilus, and Bolbosoma sp. Fatal neoplastic disease included a primary uterine T-cell lymphoma and a glioblastoma multiforme. A case of intestinal agenesis and another with multinodal heterotopic hamartomatous epithelial inclusions are reported. Direct human activity is responsible for 18% of cetaceans’ deaths, while natural pathologies would account for 82%. This long-term, pathology-based study of stranded cetaceans significantly contributes to baseline knowledge on cetacean pathology.

**Poster Number:** N-17

**Section:** Natural Disease  
**Keyword:** Infectious Disease

**FATAL H1N1 INFLUENZA IN A CANADIAN CAT**

C.G. Knight¹, J.L. Davies¹, T. Joseph², S. Ondrich³, and B.V. Rosa⁴  
¹Faculty of Veterinary Medicine, University of Calgary, AB, Canada, ²Animal Health Centre, Ministry of Agriculture, Abbotsford, BC, Canada, ³Varsity Veterinary Clinic, Calgary, AB, Canada, ⁴Independent, Calgary, AB, Canada

A 5 year old domestic cat presented for necopsy in 2014 after being found dead at home. Five days previously, a littermate living in the same home had been euthanized for acute respiratory distress.

At necropsy both lungs were consolidated and edematous. There were no other gross abnormalities in any organ system. Formalin-fixed slabs of lung had prominent pale cuffs around bronchioles that, histologically, were due to severe type II alveolar pneumocyte hyperplasia. Other histologic changes included widespread necrosis or attenuation of bronchiolar epithelium, abundant fibrin and numerous inflammatory cells (primarily macrophages) within bronchiolar and alveolar lumens and patchy alveolar septal fibrosis.

A commercial diagnostic laboratory’s feline upper respiratory disease panel was negative by real time PCR for feline calicivirus, Chlamyphila felis, feline herpesvirus 1, Bordetella bronchiseptica, Mycoplasma felis and H1N1 influenza virus. However, due to the strong suspicion for influenza, lung samples were submitted to a second laboratory for testing. Samples were positive for influenza A virus by real time PCR and typing revealed swine influenza pH1N1-09, the same strain responsible for the 2009 influenza pandemic.

This is the first reported case of pH1N1-09 influenza virus infection in a cat in Canada. Although the source of the infection is unknown, thirteen days prior to the cat’s death a houseguest had flu-like symptoms; thus, it is possible that this represents human to cat transmission of the pH1N1-09 influenza virus. This case highlights the importance of considering influenza virus infection in the differential diagnosis for respiratory distress in cats.

**Poster Number:** N-18
DISSEMINATED ALIMENTARY MYCOBACTERIOSIS IN THE HORSE- A RETROSPECTIVE STUDY OF NINE CASES

S. Hahn
Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, USA

Intestinal mycobacteriosis in horses has been sporadically reported. This case series describes intestinal and systemic infection in nine horses that were presented to the university of Helsinki teaching hospital with clinical symptoms of anorexia, weight loss, fever and diarrhea. Clinicopathological abnormalities included hypoalbuminemia and hyperfibrinogenemia. Rectal biopsies were available from five horses; Granulomatous proctitis was occasionally diagnosed, but Ziehl Neelson stain was positive in only one case. Liver biopsy was available from one horse and was positive in ZN. Attempted medical treatments failed, and all horses were euthanised. The main findings at necropsy were emaciation, severe granulomatous typhlocolitis, hepatitis and lymphadenitis in all horses. Histopathology confirmed the presence of acid fast intracellular bacilli using ZN stain. Other organs were variably involved. Mycobacterium avium ssp. hominisuis was cultured from organs collected at necropsy in five horses.

EXPLORING A ROLE FOR VIRUSES IN FELINE URINARY TRACT DISEASE

D. Goldsmith, F. Delacruz, and P. Pesavento
University of California, Davis, CA

Urinary tract disease is prevalent and increases with age in cats. Two common syndromes are chronic interstitial renal disease (CKD) and lower urinary tract disease (FLUTD). Based on serum creatinine, CKD is detectable in 50% of aged cats. The two leading causes of CKD in humans, diabetes mellitus and hypertension, do not significantly contribute to onset or progression of feline CKD. FLUTD is encountered in cats of all ages; approximately a half million cats are affected annually within the US and the majority of cases are idiopathic. In each of these syndromes, the character of the inflammation (CKD, multifocal, lymphocytic and plasmacytic), progression with age, and their associations with stress (FLUTD) or immunosuppression (CKD) suggest a potential viral etiology. In particular, both CKD and FLUTD are observed comorbidities with feline immunodeficiency virus infection. Among known feline viral pathogens, several (calicivirus, herpesvirus, foamy virus, and enteric coronavirus) have been investigated as potential causes of urinary tract disease. Most recently, a novel feline morbillivirus (FmoPV) was epidemiologically associated with CKD. We use metagenomics as a non-biased discovery tool to identify novel viral pathogens associated with urinary tract disease in California cats. In addition, we use PCR primers to the L-gene of FmoPV in a subset of animals and have detected FmoPV in feline urine. This is the first report of FmoPV isolation in the US. We also report the prevalence of FmoPV in
urine and kidney tissues in diseased and disease free cats to establish potential association with CKD or FLUTD.

Poster Number: N-20

Section: Natural Disease
Keyword: Liver and Pancreas

PAPILLARY MUCOSAL HYPERPLASIA OF THE GALL BLADDER IN FIVE CATS

A. Talley and J. Cullen
North Carolina State University, Raleigh, NC

Hyperplasia of the gall bladder mucosa has been described in a variety of species including humans, dogs, and sheep. Here we describe severe hyperplasia of the gall bladder mucosa in five cats. In these cases, mucosal hyperplasia occurred in the context of one more disease processes including suspected or confirmed bacterial cholecystitis, cholelithiasis, and pancreatitis causing extrahepatic bile duct obstruction. In all cases, there was marked papillary to cystic hyperplasia of gall bladder mucosa with variable epithelial dysplasia. Mucosal changes were accompanied by minimal to marked neutrophilic to lymphocytic inflammation, luminal mucus accumulation, and gall bladder rupture in one case. The liver was also evaluated in each case and the most consistent histologic feature was hyperplasia of small intralobular bile ducts consistent with cholestasis. Additional hepatic changes included a combination of neutrophilic or lymphocytic cholangitis, periportal edema, and hepatocellular steatosis. While these are common lesions identified in liver biopsies from cats with elevated liver enzymes, histologic changes in the gall bladders of the same patients are not frequently documented likely due to the infrequency of gall bladder biopsy or cholecystectomy in cats. Considering the relatively high prevalence of hepatobiliary disease in cats, we suspect that mucosal hyperplasia of the gall bladder as described here may be an under-diagnosed and potentially clinically significant disease process. Also, with the data compiled in this report, we propose a possible mechanism of epithelial proliferation related to the chronic inflammatory stimulation of ascending bacterial infection, cholelithiasis, extrahepatic bile duct obstruction, or a combination of these.

Poster Number: N-21

Section: Natural Disease
Keyword: Wildlife

ACARÁS (GEOPHAGUS BRASILIENSIS) AS AN ENVIRONMENTAL POLLUTION BIOINDICATOR OF THE BILLINGS DAM, SÃO PAULO: PATHOLOGY ANALYSIS OF GILLS AND HEPATOPANCREAS

A.C.C. López 1, V.L.G.S. Paiva1, I.T. Gomes1, B. Held2, G.A. Quinaglia2, and L.R.M. Sá3
Pathology of fish can be used as a biomarker for water quality and the effect of polluting agents on the aquatic ecosystem. We evaluated acara fish (Geophagus brasiliensis) as a possible bioindicator of environmental pollution, using pathology analysis of gills and hepatopancreas. Thirty males and 21 females of acara fish caught in the Billing’s dam were evaluated for general condition and pathology analysis. The animals average weight was 141,2 ± 49,06g, average standard length was 15,03 ± 2,36cm and average total length was 18,67± 2,09cm. Eleven fish (21,5%) showed none to little fat deposits, while 18 (35,2%) had adequate and 5 (9,8%) showed extensive fat deposits. Thirty nine (76,5%) fish had gills of normal color. Histopathologically, the gills of 19 (37,2%) fish presented mild congestion and edema, 8 (15,6%) showed secondary lamellae fusion, 17 (33,3%) displayed epithelial hypertrophy and hyperplasia, and 9 (17,64%) exhibited protozoan parasites. In hepatopancreas, 11 fish (21,5%) showed hepatomegaly, 27 (52,9%) presented yellow discoloration, 20 (39,21%) had a friable consistency and 6 (11,76%) showed fat marbling. Microscopically, 2 fish (3,92%) exhibited nematode parasites in the liver; 13 (25,5%) showed hepatic congestion, 16 (31,37%) exhibited hepatocyte tumefaction, 36 (70,58%) had mild to moderate lipidosis and 28 (54,9%) showed brownish pigment deposits. In the pancreas, 27 fish (52,94%) presented mild to moderate adipose infiltration. Pathology analysis of acara fish suggests that this species is a good bioindicator since several fish with a good physical condition, exhibited a high rate of hepatic lipidosis and pancreatic adipose infiltration; both considered biologically and toxicologically relevant indicators of adverse environmental conditions.

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**Poster Number:** N-22

**Section:** Natural Disease

**Keyword:** Primates

SEVERE SIMIAN IMMUNODEFICIENCY VIRUS INFECTION IN A SOOTY MANGABEY

D. Machiah, G. Smith, and P. Sharma
Divisions of Pathology and Animal Resources, Yerkes National Primate Research Center, Emory University, Atlanta, GA

Natural hosts of simian immunodeficiency virus (SIV) infection include chimpanzee, gorilla, African green monkeys and sooty mangabeys (SM). Though the SM-derived SIVsmm, is the origin of the human immunodeficiency virus type 2 (HIV-2) epidemic, the natural SIV hosts tend to have benign infections, with rare progression to simian AIDS. The mechanisms underlying this favorable outcome in SMs are not completely understood. Here-we report a 22 year old, male SM who presented for spontaneous diabetes mellitus, renal failure and chronic weight loss at the Yerkes National Animal Primate Research Center. He was serologically positive for SIV. A day before necropsy, he was reported for crusty, erythematous dermatitis on the head, thorax and abdomen and severe scrotal and hindlimb edema. Serum chemistry showed hyperglycemia, elevated hepatic enzymes, and electrolyte imbalance. Due to the worsening condition, euthanasia was elected. No significant gross lesions were
apparent at necropsy. Histopathology findings included-moderate to severe diffuse lymphoplasmacytic to granulomatous enteritis and colitis; severe diffuse pancreatic islet cell amyloidosis; severe multifocal hepatic lipidosis and multifocal lymphocytic dermatitis with hemorrhage and edema. Gram, Gomori methenamine silver and acid fast stains did not reveal any pathogens. Immunohistochemistry and in situ hybridization for SIV revealed numerous SIV positive multinucleate cells in the lamina propria of both small and large intestine. Disseminated SIV infection in a SM has been reported only once before and our case adds to the existing thought that SIV infection in natural nonhuman primate hosts is not essentially nonpathogenic, but is rather nonprogressive.

Poster Number: N-23

Section: Natural Disease
Keyword: Neoplasia

SPONTANEOUS NEOPLASMS IN VIRGINIA OPOSSUMS (DIDELPHIS VIRGINIANA): A RETROSPECTIVE CASE SERIES (1989-2014)

J. Pope and R. Donnell
Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN

This project summarizes the incidence and type of neoplasms in Virginia opossums (Didelphis virginiana) that presented to the University of Tennessee College of Veterinary Medicine (UTCVM) necropsy service between the years of 1989-2014. Eighty-five Virginia opossums were identified from the UTCVM case database and of those submitted, 17 cases from 12 patients were identified that had a diagnosis of neoplasia. These cases included 8 females, 2 males, and 2 neutered males. All opossums were greater than 2 years old. The incidence of neoplasia was 14% (12/85). Pulmonary tumors, specifically bronchioalveolar carcinomas were the most common diagnosis accounting for 53% (9/17) of the neoplasms. Additional tumors included acute myeloid leukemia with eosinophil maturation, hepatic hemangiosarcoma, sarcoma (unknown origin), squamous cell carcinoma, disseminated mast cell tumor, trichoblastoma, thyroid adenoma, and an osteoma. These findings serve as a reference for the type and frequency of spontaneous neoplasms in Virginia opossums, and based on these findings neoplasia should be considered as a differential for clinical signs in older Virginia opossums.

Poster Number: N-24

Section: Natural Disease
Keyword: Urinary System

EXPRESSION OF PHOSPHOLIPASE A2 RECEPTOR IN NORMAL GLOMERULI AND MEMBRANOUS NEPHROPATHY IN DOGS

G. Sugahara, J. Kamiie, T. Mineshige, and K. Shirota
Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan
Phospholipase A₂ receptor (PLA₂R) is expressed in the glomerular podocytes of normal human kidney but minimal or absent in murine and rat podocytes. PLA₂R is implicated as a causative antigen of human idiopathic membranous nephropathy (MN), and enhanced glomerular expression is reported in most patients of the disease. Our objective is to confirm the expression of PLA₂R in normal canine podocytes and affected glomeruli of MN cases.

Renal tissues and primary cultured podocytes were obtained from normal dogs. Using these samples, immunofluorescence, RT-PCR and sequencing, western blotting and in situ hybridization were performed to detect normal expression of PLA₂R. Immunofluorescence was performed for renal biopsy samples of eight MN cases.

Expression of PLA₂R in the glomeruli and primary cultured podocytes from normal dogs was detected, and canine PLA₂R showed high homology with human PLA2R. Immunofluorescence for normal kidney also revealed co-localization of PLA₂R with podocin, a podocyte marker located on cell membranes of the podocyte foot process. Two among eight MN cases showed increased staining for PLA₂R in the glomeruli. However, this increased expression was not observed in the glomeruli other six MN cases and other glomerular diseases.

In this study, we demonstrated PLA₂R expression in the glomerular podocytes of normal dogs. This new insight suggests that dogs can be used to establish an experimental model of human idiopathic MN. Also, increased expression of PLA₂R in the glomeruli in some MN cases may indicate the possibility of PLA₂R as a candidate autoantigen in canine MN as in human idiopathic MN.

Poster Number: N-25

Section: Natural Disease
Keyword: Neoplasia

SPONTANEOUS REPRODUCTIVE PATHOLOGY IN GUINEA PIGS

T. Veiga-Parga and S.J. Newman
Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN

Reproductive pathology in domestic guinea pigs is under-reported. To provide a comprehensive review of uterine disease in guinea pigs, a retrospective study of the pathology archives of the University of Tennessee College of Veterinary Medicine was performed. Uterine tumors were characterized by histology and 12 of 25 uterine lesions were neoplastic (48%), the other 13 lesions included cystic endometrial hyperplasia (53.8%; 7/13), mucometra (2/13; 15.3%), hemorrhage (2/13; 15.3%), uterine torsion (1/13; 7.69%), and pyometra (1/13; 7.69%). The most common uterine tumors were adenomas, representing 33.3% of guinea pig uterine neoplasms (4/12), followed by leiomyomas (25%; 3/12), and leiomyosarcomas (16.6%; 2/10). Other neoplasms included two choriocarcinomas and an endometrial polyp. In four animals, more than one tumor/lesion was reported. Five of the animals with uterine tumors and five of the animals with cystic endometrial hyperplasia also had ovarian cysts. Immunohistochemistry for cytokeratin and smooth muscle was performed yielding expected
results. Prolactin staining of the choriocarcinomas confirmed their origin. These data should serve as a reference for the types and relative frequencies of uterine pathologies in guinea pigs.

**Poster Number:** N-26

**Section:** Natural Disease  
**Keyword:** Bone and Joint

**AN OUTBREAK OF TOE-TIP-NECROSIS IN ANGUS FEEDLOT CATTLE**

G. Rimoldi and P.C. Blanchard

Two, 8-month-old Angus heifers in good nutritional status were euthanized and submitted for necropsy with a herd history of rear leg lameness, swelling and occasional sloughing of hooves. Twenty five out of a group of 100 animals moved from pasture to feedlot two weeks prior were affected. In both heifers, the lateral toe of the right rear leg had small cracks, approximately 5 mm long on the hoof’s white line, with minimal separation between the dorsal and solar aspects of the hoof. In one animal, longitudinal section of the hoof and bone demonstrated a small area of necrosis in the distal third phalanx and moderate amounts of necrotic and purulent debris infiltrating the surrounding connective tissue and dissecting between the bone and the hoof. In the second animal, lesions were more severe and extensive, with swelling up to the hock, poorly demarcated necrotic foci and large amounts of fibrin and purulent material expanding the subcutis and infiltrating the digital flexors sheaths. Bacteriological cultures from the necrotic foci demonstrated environmental opportunistic bacteria. *Trueperella pyogenes*, *Proteus* sp., Coliforms were isolated aerobically. *Fusobacterium necrophorum*, Pigmented *Prevotella / Porphrymonas* sp., *Peptostreptococcus anaerobius*, *Bacteroides fragilis* group and mixed flora anaerobically. The lesions are consistent with the condition in cattle named “toe-tip necrosis”. This condition is poorly understood but believed of traumatic origin, associated with walking on concrete floors or injuries related with moving through loading ramps, alleys and chutes. The typical presentation is progressive lameness in the first weeks coming off pasture into feedlots.

**Poster Number:** N-27

**Section:** Natural Disease  
**Keyword:** Neoplasia

**CXCR4 AND ITS LIGAND CXCL12 REGULATE CANINE HEMANGIOSARCOMA CELL MIGRATION AND INVASION**

K.S. Im$^{1,2}$, A.J. Graef$^{1,2}$, J.H. Kim$^{1,2}$, and J.F. Modiano$^{1,2}$  
$^1$Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, $^2$Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA

Canine hemangiosarcoma (HSA) is a common, very aggressive and fatal disease of dogs. Because of the highly metastatic nature of HSA, many studies have attempted to establish mechanisms of regional invasion and distant metastasis. The CXCR4/CXCL12 axis plays an important role in cell locomotion and metastasis in many cancers. Therefore, we hypothesized that the CXCR4/CXCL12 axis promotes
migration and invasion of canine HSA cells. Transcriptomic and canonical pathway analyses across 12 HSA cell lines and 58 HSA whole tumor tissues identified heterogeneous expression of CXCR4 and CXCL12, which was associated with cell movement and inflammation. In vitro, we measured CXCR4 mRNA and surface protein expression in four distinct HSA cell lines. Surface expression of CXCR4 was similarly heterogeneous within and among the cell lines. In each cell line, CXCL12 promoted calcium mobilization, cell migration, and invasion that were directly proportional to surface expression of CXCR4. Furthermore, these responses were sensitive to the CXCR4 antagonist, AMD3100. These results indicate that CXCL12 potentiates migration and invasion of canine HSA cells through CXCR4 signaling. The data suggest that the CXCR4/CXCL12 axis contributes to the metastatic progression of HSA.

**Poster Number:** N-28

**Section:** Natural Disease

**Keyword:** Bone and Joint

**PYOGRANULOMATOUS INFLAMMATION IN RESPONSE TO INTERVERTEBRAL DISC RUPTURE IN A DOG**

C.G. Knight¹, J. Diaz¹,² and J.L. Davies¹

¹Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada, ²CARE Centre Animal Hospital, Calgary, Alberta, Canada

A 5 year old golden retriever dog presented for evaluation of thoracolumbar pain. MRI showed compression of the caudal thoracic spinal cord associated with an extramedullary and extradural mass. The owners declined further investigation and the dog was euthanized.

At necropsy, the epidural space ventral to the T8-T11 spinal cord contained poorly demarcated, friable, dark red to brown material corresponding in location to the MRI findings. Similar material was present in the soft tissues of the dorsal thoracic cavity, immediately ventral to the T8-T11 vertebral bodies.

Histologically this material consisted of hemorrhage, marked neutrophilic and granulomatous inflammation and fibrovascular proliferation within the epidural fat and the soft tissues ventral to the vertebral bodies. There was no evidence of neoplasia in any section and the spinal cord was histologically normal. Two intervertebral discs were degenerate and ruptured, with fragments of disc material present both dorsal and ventral to the corresponding intervertebral spaces and surrounded by areas of intense inflammation.

Special stains were applied to identify infectious organisms in the epidural and subvertebral soft tissues, but none were seen. Given their absence, we believe that this dog’s clinical signs were caused by intense inflammation in response to the released intervertebral disc material.

Experimentally, autologous nucleus pulposus is strongly irritant in dogs, pigs and hamsters when injected extradurally or subcutaneously. This case is an example of naturally occurring disc rupture causing a severe inflammatory response. This possibility should be included in the differential diagnosis for extramedullary masses within the vertebral canal.
Poster Number: N-29

Section: Natural Disease
Keyword: Mammary

EVIDENCE OF THE SICK LOBE CARCINOGENESIS THEORY IN MAMMARY INTRAEPITHELIAL LESIONS OF FEMALE CANINES WITHOUT CLINICAL SIGNS OF MAMMARY TUMORS

J. Caicedo and C. Iregui

Background: Intraepithelial lesions (IELs) are focal outgrowths of the canine mammary gland that can be considered early precursors of tumors. Early detection of IELs have improved the survival rate in women. IELs in female canines have been proposed as comparative model of IELs in human. Objective: The main objective of this work was to histologically characterize IELs in mammary glands (MG) of 50 intact females (37 biopsies and 13 necropsies) without MG tumors. Methods: Thirty-seven biopsies of the right inguinal MG (one sample) and MGs of 13 females were collected at necropsy (108 samples). Results: MG from biopsies did not have any IELs. Nineteen MGs (6 of 13 bitches) had IELs. The most frequent IELS were adenosis (14 MG, 4 dogs) and carcinoma in situ (CaIS) (12MG, 3 dogs). IELs from complex origin were also found (complex hyperplasia and complex carcinoma). Complex IELs have not been described in veterinary pathology, but have their counterparts in developed tumors. Atypical ductal hyperplasia (ADH) wasn’t observed. We suggest that ADH is not a precursor lesion to CaIS. However, the most important finding of this work was related to the presence of CaIS that arise spontaneously de Novo in many lobules in which morphologically normal acini remain without any other sign of atypical IELs. Conclusion: In our opinion, CaIS is the canine counterpart of the "sick lobe" theory in women breast carcinogenesis, which consists that several malignant foci of breast cancer appear simultaneously or asynchronously and develop within many lobes of a single patient.

Poster Number: N-30

Section: Natural Disease
Keyword: Skin

INVolVEMENT OF INTERLEUKIN 13 FOR EXPRESSION OF PERIOSTIN IN THE CANINE ATOPIC SKIN

T. Mineshige, J. Kamiie, G. Sugahara, and K. ShirotA
Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan

Canine atopic dermatitis (cAD) is a common allergic skin disease. In cAD, once allergic inflammation is triggered by exposure to allergens, skin lesion chronically persists without continuous allergen stimulation. However, the mechanisms underlying chronicity in allergic inflammation remain unknown. Periostin, secretory protein, has been highlighted for its pivotal functions by inducing proinflammatory cytokines production and epidermal acanthosis in the allergic skin.
In this study, we aimed to clarify the involvement of interleukin (IL) -13 for periostin expression in the pathophysiology of cAD. We examined the localization of periostin and periostin producing cells in canine atopic skins. Furthermore, we focused on IL-13 which was assumed to be inducing factor of periostin, using both skins of cAD and culture cells.

Atopic skin characteristically showed intense expression of periostin in the superficial dermis. The severity of epidermal acanthosis and CD3+ cell number in the dermis was correlated with distribution pattern of periostin in the atopic skin. In situ hybridization (ISH) showed that fibroblasts and keratinocytes were the main source of periostin in cAD. By double-labeled ISH, IL-13 mRNA positive lymphocytes were detected near the keratinocytes and fibroblasts expressing periostin mRNA. By in vitro assay, recombinant IL-13 induced the gene expression of periostin in both canine dermal fibroblasts and keratinocytes.

These data suggested that IL-13 stimulated both keratinocytes and fibroblasts to produce periostin, which deposited in the canine atopic skin. Correlation between distribution patterns of periostin and histological severity in the atopic skin suggest involvement of periostin in the pathophysiology of cAD.

Poster Number: N-31

Section: Natural Disease
Keyword: Neoplasia

GLOBAL GENE EXPRESSION PROFILE IN CANINE MAMMARY CARCINOMA

Sao Paulo State University, AC Hospital

Mammary tumor is the most prevalent tumor in female dogs, presenting an important clinical problem. Gene profile studies allow the better understanding of the tumorigenic process. The aim of this study was to evaluate global gene expression profile from natural occurring canine mammary gland tumors. We evaluated a large-scale gene expression profile using fresh samples of canine mammary tissues divided into three groups: normal mammary glands (N=6), carcinomas in mixed tumor (CMT, N=15) and simple carcinomas (SC, N=15), composed by tubular carcinoma (N=7) and papillary carcinoma (N=8). Global analyses were done through Affymetrix platform (CanGene 1_0-st) and statistical analyses were performed by the Software Transcriptome Analysis Console. We observed that 1075 genes were differentially expressed between normal mammary glands and SC with 598 up-regulated genes and 477 genes down-regulated, while CMT presented 1033 genes differentially expressed, with 514 up-regulated genes and 519 genes down-regulated, when compared to normal mammary gland. Among these genes we identified TIMP2 and WNTSA, differentially expressed in both tumors types when compared to normal mammary gland. Comparing global expression from SC and CMT we found two up-regulated genes and 23 down regulated. Our results suggests that there are a great number of genes involved in canine mammary gland carcinogenic process, when compared to normal tissue, but there was no molecular significant difference between SC (papillary and tubular carcinomas) and CMT, that could explain the similar biological behavior of these tumors, with less aggressiveness and low metastatic rate.
MULTICENTRIC AND METASTATIC EMBRYONAL RHABDOMYOSARCOMA IN A YELLOW CROWNED AMAZON (AMAZONA OCHROCEPHALA) - CASE REPORT

M.D. Ronderos¹, P. Barato¹²³, B. Doncel², and J.C. Ospina³

¹Corporación Patología Veterinaria CORPAVET, Veterinary Anatomic Pathology Division, Bogotá D.C., Colombia, ²Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, National University of Colombia, ³Instituto Colombiano Agropecuario. ICA. Veterinary Pathology Division, Bogotá D.C., Colombia

A wide variety of neoplasms have been reported in pet avian species. However neoplasms arising of muscles are rare in pet and free-life avian species. This report describes a multicentric and metastatic embryonal rhabdomyosarcoma in a 6-year-old female Yellow-crowned Amazon (Amazona ochrocephala). The bird was presented with a subcutaneous mass on the lower back region. With history of vomiting, weakness, loss of body condition, dyspnea and died spontaneously. Necropsy revealed a yellowish, bilobulated mass almost completely replacing the levator coccygeus muscle, other one invading the right deep pectoral muscle and also multiple lung masses. Histopathologically, the masses were composed of sheets of oval to spindle-shaped cells with pleomorphic nuclei, numerous bizarre mitotic figures and mononucleated or multinucleated giant cells. Masson Trichrome stain, the homogeneously positive immunolabelling for sarcomeric actin, desmin antibodies and negative immunostaining against alpha-smooth muscle actin antibody confirm the skeletal muscle origin of the neoplasm. A detailed literature review of case reports for birds of the Amazon family, showed no diagnoses of embryonal rhabdomyosarcoma in Amazona ochrocephala.

COMPARATIVE PATHOLOGY OF AGING LONG EVANS AND FISCHER 344 RATS

K. Patil¹, T. Iwata¹, V. Tryon², S. Mizumori², P.M. Treuting², and J.M. Snyder¹

¹Department of Comparative Medicine, University of Washington, Seattle, WA, ²Department of Psychology, University of Washington, Seattle, WA

The laboratory rat is a valuable model used to study basic mechanisms underlying age-related cognitive decline. Two commonly used strains are the Long-Evans (LE) and the Fischer 344 (F344) rat. The LE rat,
an outbred strain, displays variability in several measures of cognitive task performance which better mimics the spectrum of deficits seen in aged humans. LE rats are longer lived than F344 rats although less is known about age-related lesions in this strain, which may impact their use as a model. This study examined >28 month old LE (n=18) and F344 (n=14) rats to characterize neoplastic and degenerative lesions identified grossly and histologically. LE rats had 16 different types of neoplasia which affected a wide variety of body systems, and 50% of LE rats (9/18) had one or more neoplasm identified on necropsy. F344 rats had 9 distinct types of neoplasia, with all rats (14/14) having one or more neoplasm identified on necropsy. The incidence of pituitary adenomas was higher in F344 (5/14) versus LE (1/18) rats. More LE rats (5/18) than F344 rats (1/14) had cutaneous or subcutaneous neoplasia, and pododermatitis was also more common in LE rats (5/18). Chronic degenerative conditions were graded on a scale of 0-4 and were common in both strains, although LE rats had significantly lower mean cardiomyopathy scores (P<0.0001) and mean glomerulonephropathy scores (P=0.008). In addition to displaying heterogeneity in cognitive performance, LE rats also appear to have substantial diversity in age-related pathology which may be more representative of the human population.

**Poster Number:** N-34

**Section:** Natural Disease

**Keyword:** Wildlife

**MORBIDITY AND MORTALITY OF THE KOALA (*PHASCOLARCTOS CINEREUS*) IN SOUTH EAST QUEENSLAND: COMBINING PATHOLOGY AND EPIDEMIOLOGY TO UNDERSTAND THE DECLINE OF AN ICONIC SPECIES

V. Gonzalez-Astudillo¹, A.B. Schaffer-White¹, L. Valenza¹, A.J. McKinnon², R.A. Larkin², J. Henning¹, and R.E. Allavena¹

¹School of Veterinary Science, The University of Queensland, Gatton, Australia, ²Department of Environment and Heritage Protection, Queensland Government, Australia.

The koala (*Phascolarctos cinereus*) is the most iconic species of Australia’s natural heritage. However, the conservation of the southeastern Queensland subpopulation is threatened by habitat loss, and additional factors like drought, bushfires, diseases, and anthropogenic-driven trauma. Since the 1990s, the population of Queensland koalas has experienced a severe decline. To explore population threats and the causes of morbidity and mortality, a retrospective analysis expanding over 15 years (1997-2012) was conducted using data sourced from wildlife hospitals and collated by the Queensland government Department of Environment and Heritage Protection Moggill Koala Hospital. Simultaneously, a large scale prospective autopsy study (2013-2016) is being conducted to determine real time threats and to explore novel etiological agents and diseases which may be impacting the population. Results show that the majority of koalas are dead on arrival or euthanized on hospital presentation. The majority of submissions are due to urogenital and ocular *Chlamydia* spp. infections. Vehicle collisions are the main cause of trauma. Unique entities identified to date include peritoneal myxosarcoma and *Ophidascaris* spp. infection. These datasets are a valuable tool to explore long-term trends in a koala population in decline. The determination of these causes will aid in the establishment of policies or future management measures targeting the conservation of this species and its unique ecosystem.
POST-SURGICAL VOLVULUS AND INFARCTION IN AN ADULT PACIFIC WHITE SIDED DOLPHIN

S. Raverty, M. Haulena, J. Rosenberg, D. Hendrickson, J. Bailey, B. Sheehan, and M. Ivančić

An adult female Pacific white sided dolphin (*Lagenorhynchus obliquidens*) presented with an abrupt change in demeanor Sunday, May 24, 2015. She was inappetant and unresponsive to trainers, with no observed defecations. She did not respond to oral fluids or antibiotics. Within 3 days peritoneal effusion and severe, progressive fluid dilation of the stomach chambers and small bowel developed. Despite intravenous fluid and antibiotic administration, an increasing inflammatory profile with deterioration in demeanor and no passage of stool was observed. An exploratory laparotomy was conducted the evening of day 4 and evaluation of the small intestine revealed moderate intramural congestion and edema with no apparent obstruction. She recovered from surgery, but was very weak and required 24 hour a day support. There was some clinical improvement observed the following day, but she declined thereafter with a clinical pathology profile consistent with sepsis and profound inflammation. She succumbed 3 days post-surgery. Necropsy confirmed abundant serosanguinous fluid with dispersed fibrin throughout the peritoneum, pericardial sac, and pleural space. There was a 1 cm long narrowing at the proximal limit of the colon. Orad to this narrowing, the small intestine was distended with acute hemorrhage. The walls were engorged with blood and edema fluid. Diffusely, the colonic lumen was reduced in caliber with prominent mucosal folds and no contents. Mechanical ileus is the prime consideration and was potentially multifactorial. This is believed to be the first emergency laparotomy in a compromised cetacean and despite the outcome, a considerable amount of information was garnered.

CRYPTOCOCCUS GATTII TYPE VGIIA INFECTION IN HARBOR SEALS (*PHOCA VITULINA*) IN BRITISH COLUMBIA, CANADA


Since first isolated in clinical case material in the late 1990’s on Vancouver Island, British Columbia, the incidence of *Cryptococcus gattii* in western Canada is now among the highest world-wide. *C. gattii* has been documented in a variety of wildlife and domestic species and has been implicated as the cause of pneumonia in stranded marine mammals, specifically cetaceans. Despite intensive recovery efforts, no cases of *C. gattii* have previously been identified in pinniped species. There is, however, one report of *C. albicus* infection in a California sea lion (*Zalophus californianus*). This report documents *C. gattii* VGIIa in
an adult male harbor seal and an unrelated harbor seal pup (*Phoca vitulina*). Both animals presented to Vancouver Aquarium’s Marine Mammal Rescue Centre with generalized weakness, dehydration, respiratory compromise, minimally responsive mentation and suboptimal body condition were apparent on initial exams. Within three days after being admitted, the 3-week old pup passed away with a large volume of thick mucohemorrhagic discharge from the nares. Radiographs of the adult male disclosed patchy pulmonary consolidation but ultrasound did not detect thoracic effusion. He succumbed during a seizure on the second day post-admission. Necropsy and histopathology findings were consistent in both animals and featured generalized lymphadenopathy, bronchopneumonia, meningoencephalitis and fungemia with intralesional yeast. Cryptococcal serum antigen levels were >1:1024. Fungal culture of lung and lymph node confirmed heavy growth of *C. gattii* type VGIa in both animals. The implications of *C. gattii* infections for pinniped population health within the region have not yet been determined.

**Poster Number:** N-37

**Section:** Natural Disease  
**Keyword:** Reproductive System

**EXTRACELLULAR VESICLES AND SMALL RNA OF THE CERVICOVAGINAL COMPARTMENT IN MACAQUES**

D. Muth, M. McAlexander, L. Ostrenga, G. Hancock, N. Pate, B. Karim, J. Izzi, R. Adams, S. Beck, K. Pate, and K. Witwer  
Department of Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine, Baltimore, MD

Macaques are an excellent model for human disease. Comparatively little is known about extracellular vesicles (EV) and their RNA cargo in the cervicovaginal compartment, especially in macaques. In this study, we compare EV and RNA in cervicovaginal secretions and cervicovaginal lavage (CVL) of rhesus and pigtailed macaques, and report on a case study of a macaque with endometriosis. Four total cervicovaginal lavage (CVL) and vaginal swab (VS) samples from rhesus and pigtailed macaques were utilized. As in our work with archived human CVL, miRs-223 and -186 were among the most abundant in CVL of rhesus and pigtailed macaques. Of more than 60 miRNAs detected consistently in all samples, only miR-29b and miR-184 were approximately as abundant in the EV as in total CVL. U6 was present almost exclusively in whole CVL. On average, 1% of CVL miRNA copies were found in EV but 2% of VS, likely due to better liberation of EV from mucus during VS swab processing. No differences were apparent in RNA expression in CVL or VS fractions from the two macaque species, although minor expression differences were observed at the tissue level, interestingly in levels of numerous let-7 family members. Comparison of particle numbers in the various reproductively healthy samples and in an individual rhesus with severe endometriosis revealed lower particle count in the diseased animal. Additional controlled studies of cervicovaginal EV and their RNA cargo are merited to characterize the potential role of EV and specific small RNAs as markers for reproductive tract disease.

**Poster Number:** N-38

**Section:** Natural Disease  
**Keyword:** Primates
PHYLOGENETIC ANALYSIS OF *HISTOPLASMA CAPSULATUM* VAR *DUBOISII* IN BABOONS FROM ARCHIVED FORMALIN-FIXED, PARAFFIN EMBEDDED TISSUES

M. Hensel¹, C.M. Smith², M. Gonzales³, A. Rodrigues Hoffmann¹, M.A. Owston³, and E.J. Dick, Jr.³

¹Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, ²Department of Veterinary Integrative Bioscience, Texas A&M University, College Station, TX, ³Texas Biomedical Research Institute, San Antonio, TX

Histoplasma capsulatum var. duboisii infections have been well documented as a cause of chronic granulomatous disease, mainly involving the skin of baboons and humans, and primarily described in African countries. While histomorphologic features are typically adequate for diagnosis given the distinct morphology of *H. capsulatum* var. *duboisii*, recent advances in molecular analysis have allowed for more specific identification of fungal species to the subspecies level and identification of phylogeographic clades. The objective of this study was to classify and develop a phylogenetic tree utilizing DNA sequences extracted from formalin-fixed, paraffin embedded (FFPE) tissues from 9 baboons from a research colony in Texas histologically diagnosed with *H. capsulatum* var *dubosii*. DNA was extracted using the MO BIO FFPE DNA extraction kit. Polymerase chain reaction (PCR) amplification followed by DNA sequencing was performed using primers targeting the fungal ITS2 region, and two *H. capsulatum* protein encoding genes, Tub1 and Arf. Sequence analysis followed by BLAST using a fungal database revealed that 9 of 9 cases matched *H. capsulatum* var *dubosii*. DNA sequences from Tub1 and Arf were edited, aligned and concatenated using Molecular Evolutionary Genetics Analysis (MEGA6) software. The resulting sequences were compared with 82 human *H. capsulatum* strains representing the eight clades of *H. capsulatum* to develop a phylogenetic tree. Based on the sequence analysis, *H. capsulatum* var *dubosii* isolated from the baboon colony most closely aligns with the African clades.

**Poster Number:** N-39

**Section:** Natural Disease

**Keyword:** Infectious Disease

GAMMA-DELTA T CELL RESPONSES IN SUBCLINICAL AND CLINICAL STAGES OF BOVINE *MYCOBACTERIUM AVIUM PARATUBERCULOSIS* INFECTION

S. Albarrak, R. Waters, J. Stabel, and J. Hostetter
Department of Veterinary Pathology, Iowa State University, Ames, IA, USDA - ARS, Ames, IA

The early immune response to *Mycobacterium avium subsp. paratuberculosis* (MAP) in cattle is characterized by a Th1-like immune response effective in controlling bacterial proliferation during the subclinical stage of infection. In young calves nearly 60% of circulating lymphocytes are γδ T cells. We hypothesize that γδ T cells promote protective immune responses in the early stages of MAP infection in cattle. Bovine γδ T cells are divided into subsets based upon their expression of Workshop Cluster 1 (WC1). In this study we evaluated WC1 γδ T cells in peripheral blood and in the ileal mucosa of cattle.
naturally infected with MAP. Cattle were in the subclinical or clinical stages of disease. Our data demonstrate that antigen specific proliferation of peripheral blood WC1⁺ γδ T cells was detected in subclinically, but not clinically infected cattle. The proliferating γδ T cells were composed of both WC1.1⁺ and WC1.2⁺ subtypes. When we used immunofluorescent staining on sections of ileum we did not observe significant difference in WC1⁺ γδ T cell subset distribution among control cattle or cattle in the sub-clinical or clinical stages of MAP infection. In all animals WC1.2⁺ γδ T cells were the predominate WC1⁺ subset in the lamina propria of the ileum. These data provide insight into the responses and distribution of WC1⁺ γδ T cells in peripheral blood and intestinal mucosa in subclinical and clinical stages of MAP infection in cattle.

**Poster Number:** N-40

**Section:** Natural Disease
**Keyword:** Wildlife

**PATHOLOGY OF KILLER WHALES STRANDED IN THE EASTERN PACIFIC BETWEEN 2004 AND 2013**

S. Raverty, J. St. Leger, K. Burek-Huntington, D. Rotstein, and J.K. Gaydos

Killer whales are the most widely distributed marine mammals in the world and in the Pacific Northwest are intimately associated with the natural history and folklore of the region. After an increase in population numbers from 1984 to 1994, there was a sudden 22 percent decline in the southern resident population between 1995 and 2002. To place this in context of other killer whale populations in the Pacific region, a review of recorded strandings from 1925 to 2011 was undertaken. A lack of comprehensive necropsies conducted during the 1900s prompted development of killer whale necropsy protocols. As a result of increased funding and public awareness between 2004 and 2011, 53 stranded killer whales were reported and examined. Diagnoses were confirmed in 22 (42%) and all age classes were represented, including: 1 abortion, 13 calves, 8 subadults, 26 adults and 3 unknowns. In those cases with pathology, infectious (6/22), metabolic (6/22), nutritional (5/22) and trauma (5/22) entities were significant contributors to morbidity and mortality within the stranded cohort. Anthropogenic factors including fisheries interactions and ship strikes were identified in 5 animals. Congenital anomalies included micrognathia inferior, hiatal hernia and cervicothoracic spina bifida. Killer whales in the northeastern Pacific are among the most contaminated marine mammals in the world and increased nutritional stressors related to declining salmon returns for the fish-eating ecotypes may result in loss of individual animals as well as to the lack of recovery of the southern resident population due to complex, multifactorial processes.

**Poster Number:** N-41

**Section:** Natural Disease
**Keyword:** Wildlife
PATHOLOGIC FINDINGS IN RAPTORS NATURALLY INFECTED WITH TWO NOVEL HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES, USA, 2014-2015

V.I. Shearn-Bochsler, S. Knowles, D.E. Green, T. Baszler, and H.S. Ip

In December 2014, novel Eurasian lineage highly pathogenic avian influenza (HPAI) A (H5N8) and reassortment H5N2 viruses were detected by the US Geological Survey National Wildlife Health Center (NWHC) in wild waterfowl carcasses recovered during a mortality event in Northwest Washington, USA. Additionally, four captive-reared gyrfalcons (Falco rusticolus) and gyrfalcon-peregrine falcon hybrids (Falco rusticolus x Falco peregrinus) that were fed meat from an American widgeon (Anas americana) shot by a hunter during this mortality event subsequently became ill and died or were euthanized. Highly pathogenic avian influenza H5N8 virus was identified from multiple tissues in all four falcons by the NWHC and the Washington Animal Disease Diagnostic Laboratory. All identifications were confirmed by the US Department of Agriculture National Veterinary Services Laboratories in Ames, Iowa. Since that time, the HPAI H5N8 and H5N2 viruses have spread to sixteen US states, killing large numbers of domestic poultry. Here we report pathologic findings of fatal avian influenza in eight wild raptors from four states received by the NWHC between December 2014 and April 2015. The eight raptors represent a wide diversity of species, including a Snowy Owl (Bubo scandiacus), a Great-horned Owl (Bubo virginianus), a Bald Eagle (Haliaeetus leucocephalus), two Red-tailed Hawks (Buteo jamaicensis), two Cooper’s Hawks (Accipiter cooperii) and a Peregrine Falcon. Gross and histological abnormalities included necrotizing encephalitis, myocarditis, interstitial pneumonia, and splenic, hepatic and pancreatic necrosis. Some raptors had myocarditis without significant encephalitis. Highly pathogenic avian influenza has the potential to significantly impact regional raptor populations in the USA.

Poster Number: N-42
Section: Natural Disease
Keyword: Avian

NECROTIZING MYOCARDITIS SECONDARY TO LISTERIA MONOCYTGENES IN AN AMERACUCANA BANTAM CHICKEN

H. Daverio¹, J. Jagne², E.A. Buckles¹, and A.D. Miller¹
¹Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, ²Department of Population Medicine, Cornell University College of Veterinary Medicine, Ithaca, NY

A 3 year-old, female, Ameraucana bantam chicken presented for necropsy with a history of weight loss, listlessness, and mouth gaping prior to euthanasia. Gross examination revealed that approximately 90% of the right ventricular free wall and interventricular septum were necrotic and the pericardial sac was distended with fibrinohemorrhagic effusion. Histologic examination confirmed a locally extensive region of necrotizing myocarditis infiltrated by abundant heterophils and macrophages. Although no definitive bacteria were noted on histologic examination; Listeria monocytogenes was isolated on bacterial culture of the pericardial effusion and immunohistochemistry for L. monocytogenes subtypes 1 and 4 confirmed
immunoreactivity within the cardiac lesion. No lesions were noted in the central nervous system. Multicentric lymphoma involving the spleen, liver, and lungs was present as a comorbidity. Endocarditis and/or myocarditis are atypical manifestations of listeriosis, with infrequent reports in both human and veterinary medicine. In human cases underlying immunosuppression is often implicated and recently specific strains of *L. monocytogenes* have been associated with cardiotropism. In this case, the multicentric lymphoma is postulated have caused immunosuppression that predisposed this patient to the development of cardiac listeriosis. This case further supports that listeriosis in birds can present solely as a cardiac disease.

**Poster Number:** N-43

**Section:** Natural Disease  
**Keyword:** Infectious Disease

**VEROTOXIGENIC E.COLI ASSOCIATED WITH DIARRHEA AND MORTALITY IN YOUNG CALVES**

Animal Health Laboratory, University of Guelph, Guelph, ON, Canada

Verotoxigenic E.coli (VTEC) was determined to be the cause of diarrhea or death in nine calves submitted to the Animal Health Laboratory (AHL) for postmortem or histological examination from January 2013 to December 2014. VTEC are a heterogeneous group of E.coli that express one or more Shiga toxins (stx). VTEC have important public health significance since some strains cause hemorrhagic colitis and hemolytic uremia syndrome in people. VTEC are carried subclinically in the intestine of healthy cattle, which is an important reservoir for human infection, and sporadically cause diarrhea and dysentery in young calves. In this series of cases the major clinical signs were diarrhea and sudden death in calves aged 4-30 days. The most common lesion at postmortem was enterocolitis which grossly was often mild but in some cases fibrinonecrotic or fibrinohemorrhagic intestinal exudates were present. The common histological lesion was attachment of bacilli to the apical border of enterocytes lining the small intestine or colon. E. coli isolates from the intestine (1 isolate was from feces) were genotyped at the AHL with a PCR assay that detects the intimin (eaeA), hemolysin (hlyA), and Shiga toxin 1 and 2 (stx1/2) virulence genes. The majority of isolates from these cases were positive for eaeA, hlyA, and stx1. Co-infections with other pathogens associated with calf diarrhea were common.

**Poster Number:** N-44

**Section:** Natural Disease  
**Keyword:** Alimentary

**HISTOPATHOLOGY OF FELINE TOOTH RESORPTION AS IDENTIFIED BY COMPUTED TOMOGRAPHY**

K.A. Potter, L.G. Lang, and T.E. Wilkinson
Washington State University, Pullman, WA
Tooth resorption is an important cause of dental disease in cats and is most often diagnosed by oral exam and dental radiography. Computed tomography (CT) is an imaging modality not previously evaluated for usefulness in identifying feline tooth resorption. As part of a larger study comparing CT with dental radiography, histopathology was used to verify lesions seen either on dental radiographs, CT or both. Affected teeth were fixed in 10% buffered formalin with multiple changes of decalcifying solution over 1 month, processed routinely and stained with hemotoxylin and eosin. A total of 176 teeth from 28 cat cadavers, including 1 radiographically normal tooth from each cat, were evaluated histologically. Tooth resorption was confirmed histologically in 110/176 teeth (62%), including 29 radiographically normal teeth. Of 1’0 teeth with histologic evidence of tooth resorption, 81 confirmed lesions seen by radiology, whereas 74 were also seen by CT. In 21 teeth, lesions were identified by CT and not by radiology; all lesions were confirmed histologically. Twelve of the 22 teeth (57%) were canine teeth. Histopathology was useful in confirming lesions identified by CT but not on radiographs.

**Poster Number:** N-45

**Section:** Natural Disease  
**Keyword:** Reproductive System

**THICKENING OF VASCULAR WALLS IN YEARLING RAM TESTES WITH AND WITHOUT TESTICULAR DEGENERATION**

Ross University School of Veterinary Medicine, Basseterre, St Kitts, West Indies, Lincoln Memorial University College of Veterinary Medicine, Harrogate, TN

Previous studies on degeneration in the ram testes have been reported in animals living in hot climates. Animals on the Caribbean island of St. Kitts are housed outdoors and likely experience chronic stress and high temperatures. Yearling rams from local farms were examined by ultrasound. From this population, white pinpoint, linear, and radial patterns were found upon ultrasonography and grossly after castration. In this study, we performed histopathology of the right and left testes from 28 yearling rams to further characterize the ultrasound and gross findings. Histopathological findings revealed thickening of the tunica media of small and medium caliber blood vessels. Patterns of degeneration were identified, such as seminiferous tubules with atrophy and sperm granulomas. Other findings included lymphoplasmacytic and histiocytic infiltration of the testicular interstitium. Our findings of testicular degeneration may have implications for herd fertility. Exposure to heat or toxicants as the etiology for these degenerative changes has not been determined.

**Poster Number:** N-46

**Section:** Natural Disease  
**Keyword:** Infectious Disease
RELATIONSHIP OF ANTIBODY TITER AND IL-4, IL-10, IL-12 AND IFN-γ EXPRESSION GAMMA WITH PERSISTENT LYMPHOCYTOSIS DEVELOPMENT IN BOVINE LEUKOSIS VIRUS INFECTED COWS

R. Favila de Alba¹, J. Zavaleta-Hernandez, H. Ramirez-Alvarez¹ J. Hernandez-Balderas², H.A. Martínez¹, M.A. Perez-Razo³, and L.A. Garcia-Camacho⁴
¹Departamento de Ciencias Biologicas, ²Departamento de Ciencias Pecuarias Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autonoma de Mexico

Persistent lymphocytosis (PL) is an outcome of bovine leukemia virus (BLV) infection that has been used as a marker of disease susceptibility. In order to relate antibody titers and expression of IL-4, IL-10, IL-12, and interferon gamma (IFNγ) with the observation of PL, 34 cows were grouped according to predetermined status of BLV infection and PL as follows: BLV+/PL⁺ (n=11), BLV⁺/PL⁻ (n=12) and BLV⁻/PL⁺ (n=11). Then, sera and cDNA of 2 monthly samplings were used to determine antibody titers and cytokine expression through ELISA and qPCR, respectively. The antibody titers were higher in BLV⁺/PL⁺ cows than in BLV⁺/PL⁻, showing a statistically significant direct relationship with lymphocyte counts. All cytokines were higher in the BLV negative group compared to the infected groups, being statistically significant for IL-10 and IL-12. The data suggests polyclonal IgM⁺, CD5⁺B target cell proliferation which in turn promoted high BLV titers in PL⁺affected cows, and a severe immune suppression in BLV⁺ animals. Hypothetically, such proliferation may enrich a population of Breg cells (IgM⁺, CD5⁺), suppressing Th1 and Th2 responses, and IL-10 secretion by negative feedback. In addition, the uninfected cows may possess some kind of resistance since they remained negative throughout the experiment which may be explained by IL-12-mediated secretion of IFNγ, and probably some other genetic traits such as presence of Bovine Lymphocyte Antigens (BoLA) resistance alleles. Further studies must be performed to characterize the putative Rg reg population and to confirm the presence of BoLA resistance alleles.

Poster Number: N-47

Section: Natural Disease Focused Scientific Session I
Keyword: Neoplasia

FELIS CATUS PAPILLOMAVIRUS TYPE 2 ONCOGENE EXPRESSION IN FELINE CUTANEOUS SQUAMOUS CELL CARCINOMAS

N.A. Thomson, J.S. Munday, and K.E. Dittmer
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, New Zealand

Felis catus papillomavirus type 2 (FcaPV-2) DNA is frequently found in feline cutaneous squamous cell carcinomas (SCCs) and is associated with up-regulation of the host cyclin dependant kinase inhibitor p16INK4A (p16) which may indicate that the virus is playing a role in cancer development. However, low quantities of papillomaviral (PV) DNA may commonly reside in basal cells and up-regulation of p16 can occur independent of PV infection making it difficult to exclude the possibility that the FcaPV-2 DNA found is an incidental, silent infection. Up-regulation of p16 in human PV-induced cancers is due to overexpression of the PV oncogenes. Therefore the aim of this study was to use real-time PCR to investigate the quantity and activity of the FcaPV-2 DNA present in feline cutaneous SCCs and correlate
this to immunohistochemical staining for p16. Expression of the FcaPV-2 oncogenes was found in 25 of 81 feline cutaneous SCCs. Positive p16 immunostaining was found in 80% of the oncogene expressing SCCs compared to just 34% of the non-oncogene-expressing SCCs (chi-sq, p <0.01). The mean FcaPV-2 DNA copy number in the oncogene-expressing SCCs was 32963 copies per copy of reference gene DNA which was significantly greater than that of the non-oncogene-expressing SCCs which was 14.1 copies per copy of reference gene DNA (p < 0.05). These results show that large quantities of transcriptionally active FcaPV-2 are present in a proportion of feline cutaneous SCCs and add to the evidence that this papillomavirus may contribute to cancer development.

Poster Number: N-48

Section: Natural Disease Focused Scientific Session I
Keyword: Neoplasia

PREFERENTIAL USAGE OF A SINGLE IMMUNOGLOBULIN HEAVY CHAIN VARIABLE GENE IN BOXERS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

E.D. Rout, R.C. Burnett, S.A. George, C.R. Abbott, J.A. Yoshimoto, and A.C. Avery
Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Canine B cell chronic lymphocytic leukemia (CLL) is common in dogs and shares many features with human CLL. In human CLL, immunoglobulin (Ig) gene use and mutation status are important markers for disease behavior, but these factors have not been assessed in canine B-CLL. Our objective was to characterize Ig gene use and mutation status in dogs with B-CLL. We sequenced the immunoglobulin heavy chain variable region (VH) genes from neoplastic peripheral blood B cells in 59 dogs with B-CLL. Thirteen VH genes were used. Excluding the Boxer breed, the most commonly used genes were VH44, VH62, and VH35. In non-neoplastic B cells, VH44 and VH62 are the most commonly used genes. Excluding Boxers, 73% of all genes used in the B-CLL patients had greater than 2% mutations compared to germline sequence and were classified as mutated, while 27% were unmutated (less than 2% mutations). VH41 is overrepresented in Boxers with B-CLL, but is not preferentially used in Boxers with large cell lymphoma. 55% (11/20) of Boxers with B-CLL use an unmutated VH41 gene. In contrast, VH41 is only used in 11% of non-Boxer breeds with B-CLL. These findings suggest that antigen selection may play a role in the pathogenesis of B-CLL in Boxers. These data lay the foundation to study antigen selection as a mechanism for B-CLL pathogenesis, correlate VH gene usage and mutation status with outcome in dogs, and further establish canine patients as good models for human CLL.

Poster Number: N-49

Section: Natural Disease Focused Scientific Session I
Keyword: Neoplasia

IMMUNOPHENOTYPIC AND HISTOMORPHOLOGIC CHARACTERIZATION OF 15 CAPRINE LYMPHOMAS
P.K. Kiser and C.V. Löhr
Department of Biomedical Sciences, Oregon State University, Corvallis, OR

Lymphoma has been described in individual cases for goats, but not systematically characterized. We report the immunophenotype and cellular morphology of 15 lymphomas from a previously published series of 97 caprine neoplasms obtained through the Oregon State University Veterinary Diagnostic Laboratory (Löhr 2013). Utilizing immunohistochemistry, 13/15 (86.6%) tumors were classified as T-cell lymphoma (CD3-positive, PAX-5-negative) and the remaining two tumors (13.3%) as B-cell lymphoma (PAX-5-positive, CD3-negative). Neoplastic cell morphology was assessed by nuclear size, nucleolar features, and mitotic count. Nuclear size was measured relative to red blood cells (rbc) and designated small (<1.5x rbc), intermediate (1.5-2x rbc), or large (>2x rbc). Eight out of 13 (61.5%) T-cell lymphomas had large, 4/13 (30.7%) had intermediate-sized, and 0/13 (0.0%) had small nuclei. Both B-cell lymphomas had large nuclei. Nucleolar/chromatin morphology was categorized as having: prominent nucleoli, indistinct nucleoli with dispersed chromatin, or clumped chromatin with parachromatin clearing. T-cell lymphomas with large nuclei contained all three nucleolar categories, whereas all T-cell lymphomas with intermediate-sized nuclei had indistinct nucleoli with dispersed chromatins. Both B-cell lymphomas had prominent nucleoli. Mitotic counts averaged over ten fields using a 40x objective (MA) were categorized as: low (0-5MA), intermediate (6-10MA), or high (>10MA). Twelve of 13 (92.3%) T-cell lymphomas had low, 1/13 (7.7%) intermediate, and none with high mitotic counts. Both B-cell lymphomas had low mitotic count. In summary, caprine lymphomas are largely T-cell lymphomas, mitotic counts are predominantly low, and nucleolar/chromatin morphology is variable in large T-cell lymphoma, but uniform in intermediate T-cell and in B-cell lymphomas.

Poster Number: N-50

Section: Natural Disease Focused Scientific Session I
Keyword: Infectious Disease

CHLAMYDIOSIS IN FARMED ALLIGATORS (ALLIGATOR MISSISSIPPIENSIS) IN LOUISIANA

K. Sakaguchi1, J. Nevarez1, D. Paulsen1, I. Langohr1, R. Bauer1, N. Crossland1, J. Ferracane2, B. Ritchie3, and F. Del Piero1
1Louisiana State University, Baton Rouge, Louisiana, 2Penn Vet New Bolton Center, Kennett Square, Pennsylvania, 3University of Georgia, Athens, Georgia

A disease outbreak leading to increased mortality at an alligator (Alligator mississippiensis) farm was investigated at the Louisiana State University School of Veterinary Medicine. Over forty 1.5-year-old alligators died within 48 hours in March 2015. A total of six animals were submitted for necropsy within 10 days of the mortalities. The livers of all animals were soft, orange to brown and bulging on the cut surface, with multiple pinpoint to 3 mm in diameter white to yellow foci. In one case, the liver also had multifocal firm, pale grey areas affecting 40% of the parenchyma. Five animals had mild to moderate hydropericardium and four of these had multiple pinpoint white foci on the surface of the cardiac ventricular epicardium. Four animals had moderate to severe conjunctivitis, characterized by white to yellow exudate. Histologically, most cases had moderate to severe heterophilic and histiocytic hepatitis with hepatocellular necrosis and biliary hyperplasia, myocarditis, conjunctivitis, and enterocolitis. Pneumonia, splenitis and nephritis were also observed. Chlamydia sp. was identified in all alligators by
PCR on liver, conjunctiva and/or feces. Intracytoplasmic Chlamydia antigens were detected via immunohistochemistry within mainly macrophages of the liver, heart and spleen; hepatocytes; cardiomyocytes; occasionally conjunctival and tonsillar epithelial cells; and rarely macrophages in the lung and conjunctiva. Further testing has ruled out infection with C. psittaci and C. pneumoniae. Attempts to culture the organism are ongoing. To our knowledge, these are first documented cases of chlamydiosis in American farmed alligators.

Poster Number: N-52

Section: Natural Disease Focused Scientific Session II
Keyword: Urinary System

EXPANDING THE SPECTRUM OF GLOMERULAR BASEMENT MEMBRANE LESIONS

E.S. Clark¹ and R. Cianciolo²
¹College of Veterinary Medicine, The Ohio State University, Columbus, OH, ²International Veterinary Pathology Service, Columbus, OH

Both podocytes and capillary endothelial cells synthesize the glomerular basement membrane (GBM), and all three components regulate glomerular permselectivity. In a subset of proteinuric dogs with Focal Segmental Glomerulosclerosis (FSGS) or minimal histologic lesions there can be non-immune complex-mediated GBM abnormalities. A retrospective review of patient data from the International Veterinary Pathology Service identified renal biopsies from 19 mature dogs, 18 of which were proteinuric. Ultrastructural and immunofluorescence evaluation of glomeruli identified multilamination and irregular club-like to villous projections of the abluminal surface of the capillary wall and an absence of immune deposits. Although this type of GBM lesion represented <5% of all specimens, review of signalment data revealed that Yorkshire Terriers were overrepresented. Specifically, 13% of biopsied Yorkshire Terriers had this lesion. Furthermore, small to medium breed dogs represented the majority of cases: 95% (18/19). Median age at the time of diagnosis was 7 years (range 3-12). Clinically, dogs presented with proteinuria (95%), elevated BUN (53%) and creatinine (74%). The most commonly identified lesions by TEM are thickened filtration membranes and podocyte foot process fusion/effacement (100%), increased mesangial matrix with mesangial hypercellularity (79%), and mesangial cell interpositioning (47%). Taken together, these data indicate that Yorkshire Terriers and other small breed dogs might be predisposed to developing GBM abnormalities that are associated with proteinuria. These lesions require TEM and IF to rule out underlying immune complex deposition. Further research is warranted to determine if these GBM lesions are hereditary defects that manifest in middle-late life or represent acquired lesions.

STP and Industrial and Toxicologic Pathology Focused Scientific Poster Presentations

Poster Number: T-1

Section: STP and Industrial and Toxicologic Pathology Focused Scientific Session I
Keyword: Respiratory System
INNATE LYMPHOID CELLS MEDIATE OZONE-INDUCED TYPE 2 IMMUNITY IN THE LUNGS OF MICE

K. Kumagai, D.N. Jackson-Humbles, R. Lewandowski, N. Li, J.G. Wagner, and J.R. Harkema
Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI

Epidemiological associations have been made between exposures to elevated ambient concentrations of ozone and eosinophilic airway inflammation in children. We have recently reported that mice repeatedly exposed to 0.8 ppm ozone develop eosinophilic rhinitis and nasal type 2 immunity that are dependent on innate lymphoid cells (ILCs). In the present study, we determined the role of ILCs in the pathogenesis of ozone-induced pulmonary lesions by using lymphoid-sufficient C57BL/6 mice, ILC-sufficient Rag2−/− mice that are devoid of T and B cells, and Rag2−/−Il2rg−/− mice that are depleted of all lymphoid cells including ILCs. Mice were exposed to 0 ppm (filtered air) or 0.8 ppm ozone for 1 day or 9 consecutive weekdays (4 h/day). Bronchoalveolar lavage fluid (BALF) was collected and lungs were processed for histopathology and gene expression analyses. All three strains of mice had increased BALF neutrophils and BrdU-positive nuclei in the bronchiolar epithelium after 1-day exposure to ozone, as compared to filtered air-exposed mice. Ozone-exposed C57BL/6 and Rag2−/− mice had increased BALF eosinophils and mucous cell metaplasia in the bronchiolar epithelium after 9-day exposure. Repeated exposure to ozone elicited overexpression of chitinase-like protein Ym2 mRNA in C57BL/6 and Rag2−/− mice. In contrast, ozone-exposed Rag2−/−Il2rg−/− mice had no pulmonary airway pathology or overexpression of transcripts related to type 2 immunity after 9-day exposure. These results in mice provide a plausible paradigm for activation of eosinophilic inflammation and type 2 immunity in the lungs of children repeatedly exposed to ozone. Research was funded by USEPA RD83479701.

§ Poster Number: T-2

Section: STP and Industrial and Toxicologic Pathology Focused Scientific Session I
Keyword: Respiratory System

EXPOSURES TO CARBON NANOTUBES AND ASBESTOS INDUCE RELATED BUT DISTINCT PROFILES OF TOXICOLOGIC LUNG PATHOLOGY

E. Frank, V. Carreira, M. Birch, and J. Yadav
Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH, CDC/NIOSH, Cincinnati, OH

Carbon nanotubes (CNTs) are rapidly emerging as occupational and environmental lung toxicants of concern due to their increasing prevalence and similarities to asbestos fibers in characteristics such as high aspect ratio and biopersistence. Because of their novelty, the long-term health outcomes of CNT exposures in humans are unknown. CNTs may become agitated into aerosols in occupational settings, posing an inhalational threat to those who work in places of CNT manufacture, distribution, and usage. However, there are limited studies or inconsistent findings regarding CNT toxicology or their toxicological similarities to asbestos. In this study, we developed mouse models of exposure using repeated, low-dose oropharyngeal aspirations of multi-wall CNTs or crocidolite asbestos. Histopathological analysis of lung sections showed that while granulomatous inflammation
was similarly induced in both exposures, CNTs caused type II pneumocyte (T2P) hyperplasia, while asbestos caused mixed-cell bronchoalveolar hyperplasia. Both exposures caused increases of fibrotic collagen as shown in Masson’s trichrome stains. Fluorescent immunohistochemistry for T2P-specific proSPC showed that T2P number was substantially increased specifically in CNT-exposed lungs. These observations are significant considering that T2P cells are known to become hyperplastic in response to alveolar epithelial injury. Co-staining for proSPC and IL-1β showed that while both exposures increased IL-1β+ cells in lung tissue, CNT-induced IL-1β increases were largely specific to T2Ps. These results illustrate that CNT and asbestos exposures may induce related but reproducibly distinct profiles of toxicologic lung pathology, and that T2Ps may be especially sensitive to CNT exposures and may be particularly relevant to CNT-induced lung injury.

**Poster Number:** T-3

**Section:** STP and Industrial and Toxicologic Pathology Focused Scientific Session I

**Keyword:** Industrial and Toxicologic Pathology

**MORPHINE DISRUPTS GUT HOMEOSTASIS AND INDUCES DISTINCT SIGNATURES OF GUT MICROBIOME AND METABOLOME PARTIALLY THROUGH THE TOLL-LIKE RECEPTOR 2 PATHWAY**

F. Wang¹, J. Meng², T. Johnson¹, C. Chen³, and S. Roy²

¹College of Veterinary Medicine, University of Minnesota, St. Paul, MN, ²Department of Surgery, Medical School, University of Minnesota, Minneapolis, MN, ³Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN

Opioids such as morphine have many beneficial properties as analgesics, however, opioids may induce multiple adverse gastrointestinal (GI) symptoms. We have recently demonstrated that morphine treatment results in significant disruption in gut barrier function leading to increased translocation of gut commensal bacteria. However, it is unclear how opioids modulate the gut homeostasis. By using a mouse model of morphine treatment, we studied effects of morphine treatment on gut microbiome, metabolome, and their interaction with host immune system. We characterized phylogenetic profiles of gut microbes, and found a significant shift in the gut microbiome and increase of pathogenic bacteria following morphine treatment when compared to placebo. Morphine treatment also resulted in dramatic changes in the fecal metabolomic profile. Through LC-MS based metabolomics profiling analysis, we identified fatty acid and bile acid metabolism to be greatly affected by morphine treatment, implicating that changes in the microbiome community has functional consequences. In a longitudinal study, we found naltrexone, an opioid receptor antagonist, reversed the effect of morphine on bile acid metabolism, indicating morphine induced changes are opioid receptor dependent. Furthermore, we confirmed that morphine induced changes were attenuated in TLR2KO but not TLR4KO mouse at day 3 post morphine treatment compared to wild type mouse, indicating opioid-receptor dependent changes are partially through TLR2 pathway. Our study shed light on effects of morphine on the microbiome-metabolome-host axis, and its role in the gut homeostasis.

**Poster Number:** T-4
Section: STP and Industrial and Toxicologic Pathology
Keyword: Reproductive System

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF UTERINE TUMORS WITH SPINDLE CELL MORPHOLOGY IN WISTAR HAN RATS

K.S. Janardhan¹, D. Malarkey², D. Dixon², C.J. Willson¹, H. Jensen², N. Clayton², and G. Flake¹
¹Integrated Laboratory Systems, Inc., Research Triangle Park, NC; ²Division of the National Toxicology Program, NIEHS, Research Triangle Park, NC

In rats, uterine neoplasms with prominent spindle cell morphology generally include endometrial stromal polyp, stromal sarcoma, leiomyoma, leiomyosarcoma and schwannoma. Histiocytic sarcoma is also a consideration when multinucleated cells are present. In one of the National Toxicology Program’s two-year bioassays, discrepant diagnoses (stromal sarcoma, schwannoma and leiomyosarcoma) for 12 uterine neoplasms from control(2) and treated(10) Wistar Han rats were discussed during pathology peer review. For one of the tumors, histiocytic sarcoma was considered. Although the tumors contained spindle cells, many tumors also contained cells with ovoid nuclei separated by stroma. Many of the tumors contained cystic spaces lined by round to low cuboidal cells, which has been considered a diagnostic feature of schwannoma. We were unable to demonstrate CD10, a human and nonhuman primate endometrial stromal cell marker, in the normal endometrial stroma of rats. However, we have observed that normal rat endometrial stromal cells stain positively with desmin and negatively with alpha-smooth muscle actin (SMA), whereas Schwann cells in peripheral nerves stain negatively with both markers. Therefore, immunohistochemistry was performed on the 12 neoplasms and a normal rat uterus using desmin, smooth muscle actin (SMA), S100, cytokeratin 18 (CK18) and Iba1 (macrophage/microglia marker) antibodies. All of the tumors were desmin positive, and many were also S100 positive. One tumor was positive for desmin, S100 and SMA (leiomyosarcoma). In addition, large numbers of Iba1 positive cells were dispersed throughout the neoplasm and normal endometrial stroma. Based upon the desmin positivity, and recognition that S100 positivity may also be seen in endometrial stromal sarcomas, we favor the likelihood that most of these sarcomas are of endometrial stromal origin.

Poster Number: T-5

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

AN ETHANOL EXTRACT OF BLACK COHOSH CAUSES HEMATOLOGICAL AND CLINICAL CHEMISTRY CHANGES CONSISTENT WITH FOLATE OR COBALAMIN DEFICIENCY IN FEMALE MICE

M.C. Cora, C. Blystone, W. Gwinn, R. Wilson, D. King, G. Kissling, S. Waidyanatha, and G.S. Travlos
Division of the National Toxicology Program, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC
Available as a dietary supplement, black cohosh rhizome is most commonly marketed as a remedy for dysmenorrhea and menopausal symptoms. Subchronic toxicity studies of black cohosh dried ethanolic extract (BCE) performed in female mice at the National Toxicology Program revealed a dose-dependent decrease in the red blood cell (RBC) count, hemoglobin concentration, and hematocrit with a dose-dependent increase in the mean corpuscular volume (MCV); reticulocyte counts were unchanged. These erythron changes indicate ineffective erythropoiesis consistent with a megaloblastic disorder. The purpose of our study was to investigate the mechanism by which black cohosh induces these erythron changes. B6C3F1/N female mice (32/group) were exposed by gavage to vehicle control (0.5% methylcellulose) or 1000 mg/kg BCE for 90 days. Terminal blood samples were analyzed for basic hematology and clinical chemistry parameters as well as for folate, cobalamin, RBC folate, total homocysteine and methylmalonic acid (MMA) concentrations. Observed hematological changes were as follows: decreased RBC count, increased MCV, and decreased reticulocyte, white blood cell, neutrophil and lymphocyte counts. Blood smear evaluation revealed increased Howell-Jolly bodies and rare basophilic stippling in the treated animals. No treatment-related effects were observed on the basic clinical chemistry panel or with folate, cobalamin or RBC folate concentrations; however, total homocysteine and MMA concentrations were increased in the treatment group. Under the conditions of our study, BCE administration causes hematological and clinical chemistry changes consistent with folate or cobalamin deficiencies.

**Poster Number:** T-6

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Cardiovascular

**MINIMAL MYOCARDIAL DEGENERATION AND INFLAMMATION IN THE RAT: ADVERSE OR NON-ADVERSE?**

R. Haworth, F. Chanut, M. York, and Y. Cui
GlaxoSmithKline, Park Road, Ware, UK

**Introduction:** Judging adversity of histopathology findings in toxicity studies is often difficult. This poster describes a case study where a myocardial finding was observed at higher incidence in treated animals but at the same grade as controls. A compound was given at three doses by nebulised solution to Sprague Dawley rats in 14 and 28 day inhalation toxicity studies. **Methods:** Standard in-life, toxicokinetic, clinical pathology (including cardiac troponin I in the 28 day study) and histopathology endpoints were evaluated. **Results:** In the 14 day study, minimal myocardial degeneration/inflammation was observed in intermediate and high dose animals in both sexes, but not in controls. In the 28 day study, this finding was observed in control and treated animals in both sexes, with a higher incidence in high dose males. Cardiac troponin I concentrations were variable with increases in 2 treated animals on day 3 and a single high dose female on day 28 only. Changes indicative of minimal irritation were observed in nasal turbinates and/or larynx in both studies. **Conclusions:** Myocardial degeneration/inflammation was observed at an increased incidence in treated animals and was considered related to test article. This interactive poster will consider the weight of evidence approach to assessment of adversity and include an opportunity for conference delegates to vote on their view of the adversity of this finding.
"All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals."

**Poster Number**: T-7

**Section**: STP and Industrial and Toxicologic Pathology  
**Keyword**: Industrial and Toxicologic Pathology

**HISTO-MORPHOLOGICAL ALTERATIONS IN ORGANS AND TISSUES OF THE AFRICAN CATFISH EXPOSED TO DEXAMETHASONE**

N. Kolawole, J.Ogunsola, and O. Adedeji

Aquaculture industry in Nigeria has witnessed tremendous growth in terms of production and operational volume. Presently, the industry is witnessing an upsurge in the incidence of disease and high cost of feed with little or no policy to mitigate such occurrence. This has led to proliferation of arbitrary use of drugs. Dexamethasone is a synthetic glucocorticoid used by farmers in Nigeria as a growth promoter to reduce feeding costs and enhance fish growth. This study examined the histological effects of Dexamethasone on some organs of the African catfish Clarias gariepinus following a 21-day exposure. A total of 240 catfish were exposed to graded concentration of the test drugs at 50, 500, and 1000µg/l in triplicate. Water quality parameters were routinely analyzed and behavior and mortality of fish were monitored. At the end of the exposure period, gills, kidney, liver and gonads were processed for histology. Results obtained show that fish exposed to Dexamethasone have marked hyperplasia of the secondary lamellae in the gills; uniformly-sized large cytoplasmic vacuoles in the hepatocytes; prominent haemopoietic compartment and depleted tubular compartment in the kidneys; seminiferous lobules were filled with densely-packed spermatogenic cells while majority of the mature ovarian follicles were distorted with wrinkled outlines. Our findings suggest that the use of Dexamethasone as a growth promoter in the catfish could result in adverse effects in various organs and possibly compromise the health of fish.

**Poster Number**: T-8

**Section**: STP and Industrial and Toxicologic Pathology  
**Keyword**: Neoplasia

**MECHANISTIC STUDIES DEMONSTRATED THAT RENAL TUBULAR AND ADRENAL TUMORS IN THE 2-YEAR RAT STUDY WITH CANAGLIFLOZIN WERE RAT SPECIFIC AND NOT RELEVANT FOR HUMAN RISK ASSESSMENT**


Canagliflozin, a non-genotoxic sodium glucose co-transporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes mellitus, increased the incidence of pheochromocytomas and renal tubular tumors in the 2-year rat study. In a prior mechanistic study based on the hypothesis that tumors were due to
carbohydrate malabsorption, it was demonstrated that replacement of the standard diet (SD) with a 40% fructose diet (FD) prevented carbohydrate malabsorption (effects on calcium metabolism and cell proliferation in kidney and adrenal).

The objective of this 15-month study was to determine if long-term feeding of FD prevented tumor formation. Male Sprague Dawley rats (90/group) were fed SD or FD and administered canagliflozin at 0 (SD), 0 (FD), 65 (FD), 100 (SD), and 100 (FD) mg/kg/day for 15 months. The following tissues were collected at necropsy and examined microscopically: adrenal glands, bone (sternum and femur) and kidneys.

Microscopically, six basophilic renal tubular tumors (4 adenomas and 2 carcinomas) were present in the kidneys of rats in the 100 (SD) mg/kg/day group, but no renal tumors were present in rats fed FD and administered 65 or 100 mg/kg/day. Adrenal medullary hyperplasia was increased (35/90) in rats administered 100 (SD) mg/kg/day and was unaffected in rats dosed at 65 and 100 mg/kg/day on FD. The incidence of pheochromocytomas was similar in all groups.

In conclusion, it was demonstrated that the kidney tumor formation and adrenal medullary hyperplasia were prevented by the fructose diet, and therefore secondary to carbohydrate malabsorption and the associated hyperabsorption of calcium.

**Poster Number:** T-9

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Liver and Pancreas

**SAFETY ASSESSMENT AND TRANSGENE EXPRESSION OF HYDRODYNAMIC GENE DELIVERY IN C57BL6 MICE**

J. Ooi, X. Chen, L. Wang, K. Kang, and J. Jin
Novo Nordisk Research Center, Beijing, China

Hydrodynamic Gene Delivery is a fairly new yet efficient method for delivering exogenous genes to murine hepatocytes. It employs a physical hydrostatic force generated by a rapid injection of a large volume of DNA solution into a into a tail vein that resulted in the hepatic sinusoidal expansion and disruption of fenestrae, and therefore enhance the permeability of endothelium and the plasma membrane of the parenchyma cells to allow delivery of DNA into cells. NNRCC have recently implemented in house HGD as a platform for both target screening and validation. Assessment of the safety and effectiveness of a hydrodynamic gene delivery method is an essential step toward this goal. In this report, we showed the time course effect and the expression of the epitope-tagged transgenes on the liver after hydrodynamic tail vein injection.

Results: All liver sections evaluated showed variable degree of sinusoid expansion and congestion at 5min. Multifocal areas of liver injury was noted in liver sections within 1wk. By 2-4wks, there were very little liver changes. When present, they were characterized by minimal to mild fibrosis, loss of hepatocytes, and aggregates of puffy macrophages. Hepatocellular expression of transgene was robust and consistent. Conclusion: HGD method was well tolerated within the duration and parameters of the study.
It resulted in a sustained, robust expression of transgene for as long as 4 weeks.

**Poster Number:** T-10

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**INGESTED SILVER NANOPARTICLES: EFFECTS ON THE INTESTINAL MICROBIOME AND PHYSICOCHEMICAL ALTERATIONS IN A SIMULATED GASTRIC ENVIRONMENT**

I.L. Bergin¹, A.P. Ault¹, L.A. Wilding¹, J.L. Axson¹, C.M. Bassis³, D.I. Stark¹, K. Walacavage¹, S.S. Capracotta², S.A. Hashway, M. Morishita¹, P.R. Leroueil¹, A.D. Maynard¹, and M.A. Philbert¹  
¹University of Michigan, Ann Arbor, MI, ²Malvern Instruments, Westborough, MA

The intestinal microbiome has been increasingly recognized as a potential target for adverse effects of toxicants. Silver nanoparticles (AgNPs) have antimicrobial effects in vitro or when applied topically, in large part because of dissolution to ionic silver (Ag+). There is concern that ingestion of AgNP, either intentionally as dietary supplements or unintentionally through dissolution from silver-coated consumer products, may adversely affect the gut microbiome similarly to a broad-spectrum antibiotic. We evaluated the effects of AgNP ingestion on the indigenous intestinal microbiome in mice gavaged for 28 days with 10 mg/kg of AgNP. AgNP were 20 or 110 nm diameter and coated with citrate or polyvinylpyrrolidone (PVP). The AgNP dose corresponded to 2000x the EPA oral reference dose and 400X the in vitro antimicrobial concentration. The murine cecal microbiome was evaluated by 454 pyrosequencing of bacterial 16S RNA genes. In contrast to an antibiotic, no significant differences in microbial community structure or diversity were associated with AgNP administration. We further evaluated physicochemical alterations of AgNP in simulated gastric fluid. Rapid increase in particle size was observed, with higher rates at lower pH. This was consistent with a model of AgNP gastric dissolution to Ag+, followed by rapid precipitation with Cl- and aggregation of AgCl particles. We suggest that the lack of antimicrobial effects is due to transformation of AgNP to aggregated AgCl particles, minimizing the biological availability of Ag+ in the distal intestine. Thus AgNP may be less potent as an antimicrobial when ingested, in comparison to topical applications.

**Poster Number:** T-11

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Cardiovascular

**ENDOVASCULAR INTERVENTIONS IN AN OVINE MODEL OF TISSUE ENGINEERED VASCULAR GRAFT STENOSIS**

**Introduction:** Tissue engineered vascular grafts (TEVG) are the first man-made grafts with growth potential and have been utilized clinically, but widespread clinical use is limited due to a high rate of stenosis. Fortunately the majority of patients with stenosis have been successfully treated with balloon angioplasty (BA) for stenosis. However, it is currently unknown how these endovascular interventions affect neo-vessel development. **Experimental Design:** We aim to duplicate TEVG stenosis in a juvenile ovine model and evaluate the effects of endovascular intervention for its management. **Methods:** Cell-free TEVG were implanted as thoracic caudal vena cava interposition grafts (n=4) for up to 75 days. Animals underwent serial angiography at 0, 30, and 60 days to evaluate graft patency and remodeling. BA was performed using a low-pressure compliant balloon catheter in cases of stenosis. Graft tissue was harvested at each time point for histologic analysis. **Results:** Stenotic TEVGs were treated with BA on post-operative days 30 and 60. Median luminal patency was 52.9% [range: 22.1-55.7%]. Pressure gradients pre-intervention were a mean of 14 mmHg [8-17 mmHg]. BA increased lumen diameters to a median of 61.4% [27.9-64.3%] and decreased mean pressure gradients by a median of 5.0 mmHg [3.5-5.0 mmHg]. Histopathology demonstrated neotissue formation, including neo-intima and neo-media as early as 35 days post-implantation. **Conclusions:** An ovine model using cell-free TEVGs recapitulates clinical TEVG stenosis, which can be successfully treated with BA during acute time points. These results will improve current clinical practice and the safety of TEVG angioplasty.

**Poster Number:** T-12

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Cardiovascular

**MICROANATOMIC DETERMINANTS OF EFFICACY OF ENDOVASCULAR RADIOFREQUENCY RENAL DENERVATION IN SWINE**

J. Keating, A. Tzafriri, and J.R.L. Stanley
CBSET, Inc., Lexington, MA

Radiofrequency (RF) renal denervation (RDN) is a treatment option for human patients with refractory hypertension, yet clinical response to first generation single electrode therapy remains highly variable and incompletely explained. We combined histomorphometry and biomarkers in the swine model with computational modeling of energy and heat transport in order to evaluate the effects of a multielectrode RF catheter and to understand drivers of variability. Multielectrode RF denervation was shown to be associated with reductions in systemic blood pressure (BP) and renal norepinephrine (NEPI) seven days after treatment, and correlated with the amount of treated nerve tissue associated with ablation zones. Efficacy of RF electrode treatments is influenced by nerve distribution patterns, such as the predictable changes in nerve location and distance that occur longitudinally along the renal artery in addition to radially distributed (i.e., ventrodorsal and craniocaudal) anatomic asymmetries. Ablation zone geometry is influenced by structures such lymph nodes and blood vessels which can draw RF power and diffuse heat, and these observations informed the development and validation of a predictive computational model of ablation geometry in catheter-based RDN.

**Poster Number:** T-13

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Industrial and Toxicologic Pathology
LONG-TERM IMPLANTATION WITH TITANIUM ALLOYS RESULTS IN A PRO-INFLAMMATORY AND PRO-THROMBOTIC STATE

G.H. Frydman¹,²,⁵, R.P. Marini¹, P. Bendapudi²,⁵, C.R. Vanderburg³,⁵, B. Lai⁴, V. Bakthavatchalu¹, R.G. Tompkins²,⁵, and J.G. Fox¹
¹Massachusetts Institute of Technology, Cambridge, MA, ²Massachusetts General Hospital, Boston, MA, ³Massachusetts General Hospital Institute for Neurodegenerative Disease, Charlestown, MA, ⁴Argonne National Laboratory, Argonne, IL, ⁵Harvard Medical School, Boston, MA

Titanium-based alloys are used in human and veterinary medicine. Recent studies in human literature suggest that titanium alloys may induce local and systemic inflammatory response. In this study, 37 rhesus macaques (Macaca mulatta) with long-term cranial titanium implants (0-7 years duration) were evaluated for changes in their inflammatory and coagulation profiles. Negative controls (n=28) did not have implants. Animals with implants had significantly higher plasma D-dimer concentrations (365.4 ± 315.2 µg/mL) compared to non-implanted animals (194.9 ± 142.93 µg/mL). Additionally, animals with implants had significantly higher immunoglobulin, lower albumin, and lower calcium concentrations (3.37 ± 0.5 g/dL, 3.71 ± 0.5 g/dL, 9.13 ±0.4 mg/dL) compared to non-implanted animals (2.72±0.3 g/dL, 4.21 ± 0.3 g/dL, 9.51 ± 0.6 mg/dL). These changes were also significantly correlated with duration of implant. Chronic purulent exudate with bacteria was observed on the skin around the implant site, and rarely within deeper tissues. Histopathology around the implant site revealed chronic pyogranulomatous inflammation extending from the skin to the dura mater. X-ray fluorescence microscopy of tissue biopsies from the implant site revealed significant increases in metal ions within the tissue, including titanium and iron. Free metal ions persisted in the tissues up to 6 months post-explant. These results suggest that long-term implantation with titanium alloys results in the leaching of metal ions into local tissues with associated inflammation and also a possible inducer of systemic pro-thrombotic and pro-inflammatory state.

§ Poster Number: T-14

Section: STP and Industrial and Toxicologic Pathology
Keyword: Neoplasia

GASTRIN-RELEASING PEPTIDE RECEPTOR (GRPR) SIGNALING IN PROSTATE CANCER

S. Elshafae¹, B. Hassan¹, W. Supsavhad¹, W. Dirksen¹, H. Ding², M. Tweedle², and T. Rosol¹
¹Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA, ²Department of Radiology, The Ohio State University, Columbus, OH, USA

The gastrin-releasing peptide receptor (GRPR) is a cancer-associated antigen that is upregulated in prostate cancer (PCa) and other solid tumors. This study explored the GRPR agonist, bombesin (BB), on proliferation, migration and epithelial-mesenchymal transition (EMT) gene expression in canine PCa Ace-1 cells and its use to image in vivo a novel dog model of Ace-1 PCa. Ace-1 cells were transduced with a functional human GRPR receptor cDNA. Clones with high expression and binding activity (IC₅₀ 5.3nM) were selected. BB enhanced cell proliferation, migration and invasion in vitro, and upregulated EMT
genes (Twist, Snail and Slug) and downregulated epithelial genes (E-cadherin and B-catenin). Blocking of GRPR signaling induced the mesenchymal-to-epithelial transition (MET) phenotype. BB increased PCa tumor growth in nude mice. A near-infrared fluorescent BB analogue was developed to image focal PCa in cyclosporine-immunosuppressed beagles after ultrasound-guided injection of Ace-1 cells into the prostate glands. The analogue was injected into the lateral prostate artery using fluorography-guided arterial catheterization. It accumulated in the PCa over 24-48 hours due to receptor internalization, but not areas of benign prostatic hyperplasia. These data demonstrate that GRPR signaling is important in PCa and targeting of GRPR is promising for imaging and treatment of PCa. Future studies will identify the downstream signaling molecules of GRPR in PCa.

**Poster Number:** T-15

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Skin

**EVALUATION OF DRUG PHOTOTOXICITY IN SKIN LAYERS BY MULTIMODAL MOLECULAR IMAGING TECHNIQUES**

P-M Vaysse, G. Hamm, A. M. Sargeant, F. Pamelard, D. Bonnet, T.N. Merriman, and J. Stauber

1ImaBiotech, MS Imaging Dept., Loos, France, 2Charles River, Spencerville, OH, USA

Phototoxicity is an inflammatory skin reaction caused by exposure to a chemical and subsequent exposure to sunlight or ultraviolet radiation. This phenomenon is mainly a concern for drugs and pharmaceuticals that are either ingested or applied directly to the skin, for example, as a cream. In this context, Mass Spectrometry Imaging (MSI) enables spatially resolved and unlabeled imaging of the drug and its photo-metabolites directly in their skin micro-environment and the discovery of new molecular biomarkers that are regulated by a photo-irradiation and are specific to skin substructures. In a proof of concept experiment, this technique was used to study the absorption, distribution and metabolism of a drug, Ketoprofen, its photo-metabolites as well as some potential biomarkers of toxicity or inflammatory process within minipig skin after photosensitization. A Quantitative measurement of Ketoprofen and its photo-metabolites was performed using a specific MSI protocol to locally evaluate the amount of each targeted molecule within skin substructures. Moreover, the distribution of some inflammatory biomarkers can be assessed and compared with the drug localization and quantification in the same histological structures of the skin. Immunohistochemical (IHC) staining was used on adjacent skin sections to follow Langerhans cells within the epidermis using CD1a labeling. MS images were then correlated with IHC results to establish a relationship between cell types and molecular species’ modulation. We present here a new approach to evaluate the phototoxicity of a drug, to discover toxicity biomarkers and to interpret biological or pharmacological mechanisms within tissues based on MS image analysis.

**Poster Number:** T-16

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Neoplasia
NOVEL TRIMODAL THERANOSTIC NANOPORPHYRINS IN AN ORTHOTOPIC PATIENT-DERIVED XENOGRAFT(PDX) BLADDER CANCER MOUSE MODEL

University of California-Davis, Sacramento, CA, The Jackson Lab, Sacramento, CA

The overall prognosis of bladder cancer has not been improved over the last 30 years. There is a great medical need to develop novel diagnosis and therapy approaches for bladder cancer. We developed a multifunctional nanoporphyrin platform that was coated with a bladder cancer-specific ligand named PLZ4. PLZ4-nanoporphyrin (PNP) integrates photodynamic diagnosis, magnetic resonance imaging, image-guided photodynamic therapy, photothermal therapy and targeted chemotherapy in a single procedure. PPNs are spherical, relatively small (around 23 nm), and have the ability to preferably emit fluorescence/heat/reactive oxygen species upon illumination with near infrared light. Doxorubicin (DOX) loaded PNNs possess slower drug release and dramatically longer systemic circulation time comparing to free DOX. The fluorescence signal of PNNs could efficiently and selectively accumulate in bladder cancer cells but not normal urothelial cells in vitro and in orthotopic patient derived bladder cancer xenograft (PDX) models, indicating their great potential for photodynamic diagnosis(PDD). Photodynamic therapy with PNNs was over 100 times more potent than 5-aminolevulinic acid, and eliminated orthotopic PDX bladder cancers after intravesical treatment. Image-guided photodynamic and photothermal therapies synergized with targeted chemotherapy of DOX and significantly prolonged overall survival of mice carrying PDXs. In conclusion, this uniquely engineered targeting PNP could not only selectively target tumor cells for PDD but also serve as effective triple-modality (Photodynamic/photothermal/chemo) therapeutic agents against bladder cancers. This platform can be easily adapted to individualized medicine in a clinical setting and has tremendous potential to improve the management of both non-myoinvasive and advanced bladder cancer in clinic.

Poster Number: T-17

Section: STP and Industrial and Toxicologic Pathology
Keyword: Nervous System

SUSPECT MELARSONINE DIHYDROCHLORIDE RELATED MYELOPATHY IN A GERMAN SHEPHERD DOG

T. Peterson¹,², A. Dedeaux³, N. Welborn³, K. Ryan³, and F. Del Piero¹,²
¹ Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA,
²Louisiana Animal Disease Diagnostic Laboratory, Baton Rouge, LA, ³Department of Veterinary Clinical Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA

Melarsomine dihydrochloride is an anthelmintic adulticide used to treat Canine Heartworm Disease (CHD). The drug is injected intramuscularly (IM), typically in the lumbar epaxial muscles. An adult, female spayed German shepherd dog with Class 2 CHD received a single IM injection of melarsomine according to manufacturer’s labeling. Post injection clinical signs included non-ambulatory paresis and restlessness, which progressed over the next 24 hours to include absent patellar reflexes, diminished
withdrawal reflexes, and minimal voluntary motor in hind limbs. Magnetic resonance imaging of the injection site (L3-4) and spinal cord revealed mild T2 hyperintensity of the epaxial muscles and incidental non-compressive disc herniation at the lumbosacral junction. The patient continued to deteriorate, showing signs compatible with hypovolemic shock and ascending myelomalacia. Humane euthanasia was elected. On gross and histological evaluation, severe filarial nematode infection was present within the pulmonary artery with severe proliferative endarteritis, and multifocal intra- and extradural hemorrhage throughout the spinal cord. Most significant histologically was a malacic focus with neutrophilic infiltration and hemorrhage, capillary thrombi, and an intracapillary microfilaria in the spinal cord at the level of L5-6. These changes are speculated to have been caused by paraspinal drug inoculation with diffusion of injection volume into the spinal canal with resulting secondary malacia. Paresis and paralysis associated with melarsomine treatment have been uncommonly reported. This case provides a unique example of suspected fatal adverse reaction related to paraspinal IM injection of melarsomine.

Poster Number: T-18

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

AN INTEGRATED OPTICAL DENSITY BASED-MEASUREMENT TO EVALUATE CISHL STAINING IN TISSUE SECTIONS

F. Aeffner1, C. Schnatwinkel1, H. Stern2, V. Villegas2, A.J. Milici1, G.D. Young1, and J.S. Krueger1

1Flagship Biosciences Inc., Westminster, CO, 2Infinity Pharmaceuticals Inc., Cambridge, MA

For many years, chromogenic in-situ hybridization (CISH), or its fluorescent counterpart (FISH) have been successfully utilized to evaluate gene alterations in various cell and tissue types. CISH has emerged as a powerful alternative to immunohistochemistry (IHC) to quantify a gene product, particularly when specific antibody reagents are not available or when the expression patterns of highly homologous proteins need to be evaluated. There is currently no well-defined scoring protocol for RNA CISH. Dot-counting is one proposed approach, but counting frequently becomes challenging due to variability in dot staining intensity, dot size, and dot frequency. This variability can impact both subjective interpretation and development of automated scoring algorithms.

We developed a tissue image analysis approach that accurately distinguishes and classifies cells according to a summary cell-based measurement of Integrated Optical Density (IOD). The algorithm incorporates dot intensity, dot size, and dot frequency for RNA CISH applications. We first demonstrated that IOD assessment of target genes in various tissues performs similar to the conventional method of counting the number of dots in an ideal setting where dot intensity, size, and frequency is relatively uniform. We then extended the IOD classification to the RNA CISH, where the variability in dot size, intensity, and frequency compromises a reliable dot number assessment.

In summary, we have developed an image analysis approach which quantifies RNA CISH staining based on the integration of dot number, dot size, and dot intensity. This method complements the commonly used dot-counting approach when dot characteristics are not uniform.

Poster Number: T-19
Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

QUANTIFYING PD-L1 SPATIAL DISTRIBUTION SIGNATURES FOR PATIENT SELECTION APPROACHES

Flagship Biosciences Inc., Westminster, CO

Inhibitors of inflammatory checkpoints (e.g. PD-L1 inhibitors) have demonstrated great promise in preclinical and clinical studies. They focus on controlling natural inflammatory checkpoints to stimulate an elevated inflammatory response against tumors through increased anti-tumor inflammatory cell infiltrates in the tumor microenvironment (TME; stroma) and tumor epithelium. Cells positive for biomarkers of inflammatory cells or mechanisms (e.g. PD-L1, CD8, etc.) are often assessed qualitatively or semi-quantitatively using immunohistochemistry in a subset of representative microscopy fields. However, the locale of inflammatory biomarkers, such as PD-L1, may be more revealing than estimating tumor-wide dispersion of biomarker-positive cells. Unfortunately, the spatial relationships and complex distribution of inflammatory cells in tissue context pose significant challenges for a meaningful manual evaluation by a pathologist.

We have developed an approach which quantified spatial relationships in tissue context. PD-L1-positive cells were quantified with CellMap™ software relative to: 1) the total number of cells in the tumor and TME tissue compartments, and 2) the number of cells within distance ranges from the tumor/TME interface. No clear sample stratification was identified with total cell analysis. However, samples could be preliminarily stratified by PD-L1+ cell density profile relative to the tumor/TME interface. This proof-of-concept study demonstrated a unique quantitative contextual assessment of inflammatory cell infiltrates in tumors that could be used to gain new insights into 1) inflammatory cell type distributions and interactions in tumors, 2) inflammatory cell spatial responses to oncology therapies, and 3) novel patient selection criteria for traditional and immuno-oncology therapeutics.

Poster Number: T-20

Section: STP and Industrial and Toxicologic Pathology
Keyword: Immune System

QUANTITATIVE ANALYSIS OF MULTIPLE SUBTYPES OF IMMUNE SYSTEM CELLS IN CANCER TISSUES

G.D. Young1, F. Aeffner1, K. Wilson1, M. Peltjo1, J. Major1, N.T. Martin1, J.S. Krueger1, H. Lange1, J.D. Alvarez2, M. Sharp2, M.A. Sepulveda2, and A.J. Milici1
1Flagship Biosciences Inc., Westminster, CO; 2Janssen R&D, Spring House, PA

Current cancer biology acknowledges the key role of the immune system in tumor biology, and modulation of immune system holds promise in cancer treatment. Molecular markers allow for reliable identification and subsequent analysis of inflammatory cell types/subtypes, and 6-8 antibody markers
are needed to classify immune cell types/subtypes. Measuring these immune cell populations in the TME may have prognostic value or predict response to a given therapeutic agent. Unbiased quantitative whole-tissue image-based approaches are needed for analyses.

In this proof-of-principle study, we used a tissue image analysis (TIA) approach to integrate spatial expression of 6 serial-section IHC stained cell-type specific markers (CD68, CD4, CD8, CD33, FoxP3 and CD11b) in whole tissue clinical lung samples to evaluate cell populations.

IHC staining followed by application of Flagship’s CellMap™ algorithm allows cell-by-cell quantification and localization of the cells in whole tissues. FACTS™ (Feature Analysis on Consecutive Tissue Sections) integrates the spatial expression of individual markers onto a reference H&E slide. MultivariateMap™ integrates the patterns of each marker based on immune cell type function and allows the visualization and measurement of multiple cell subtypes in the same tissue. Together, these approaches avoid difficult-to-implement wet assay strategies involving multiplexing 6 markers onto the same section, which may be difficult to implement in the diagnostic setting needed to support patient selection strategies. Flagship’s proprietary image analysis approaches provide a robust platform for immunoncology applications by providing information on the state of the immune system in cancer using approaches implementable in the clinic.

Poster Number: T-21

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

ESTABLISHMENT OF ROUTINE, FULLY AUTOMATED RNA IN SITU HYBRIDIZATION READOUTS OF APOPTOSIS AND PROLIFERATION-RELATED BIOMARKERS IN PRECLINICAL ANIMAL MODEL TISSUE PANELS USING RNAscope® LS ASSAY ON LEICA BIOSYSTEMS’ BOND RX

M-H He², D. Kim², T. Franks¹, M. Roy¹, C. Bunker², Y. Luo², X-J Ma², and E. Park²
¹Drug Safety Research and Development, Pfizer Global Research and Development, Groton, CT,
²Advanced Cell Diagnostics, Hayward, CA

Assessing tissue distribution of therapeutic targets and toxicity biomarkers is essential, however immunohistochemistry is often inefficient due to inconsistent performance, extensive validation time and lack of reagents. RNAscope® in situ hybridization (ISH) technology is a universal assay to detect and characterize tissue distribution of any target or biomarker mRNA. RNAscope ISH has molecular sensitivity, high specificity and robust performance in FFPE tissue. Fully automated RNAscope ISH is performed on Ventana Discovery and Leica Biosystem Bond RX instrumentation; detection of marker RNAs is achieved in 8 hours and visualized via bright field microscopy. Marker RNAs can be quantified with SpotStudio™ or other digital image analysis software.

We present RNAscope applications for detection of cell-type, proliferation and apoptosis biomarkers in animal tissues used routinely in toxicity studies. We demonstrate robust performance in 25 tissues from rat, dog and cynomolgus monkey using probes to low-, medium- and high-expression housekeeping gene RNAs (POLR2A, PPIB and UBC). Specific signals and well-maintained morphology was achieved
efficiently for all 25 tissues. Based on optimized epitope retrieval conditions, tissues were grouped into two multi-tissue microarrays for high-throughput marker analysis. We examined and present tissue-specific expression patterns of endothelial marker CD31, macrophage marker CD68, proliferation markers Ki-67 and Cyclin E1, and apoptosis molecules, Puma, Fas/CD95 and DR5.

The presentation demonstrates RNAScope as a method that can be replicated in any lab and suitable for efficient and reliable detection of any target candidate or safety/toxicity biomarker mRNA from any species for interrogation in any tissue.

Poster Number: T-22

Section: STP and Industrial and Toxicologic Pathology
Keyword: Respiratory System

INDUCED INTERSTITIAL PULMONARY FIBROSIS (IPF) MODEL: UNLABELED BIEOMYCIN DISTRIBUTION AND EARLY IPF MARKERS IDENTIFICATION BY MALDI IMAGING

D.Bonnel¹, M. McElroy², E. Falaux¹, G. Picard de Muller¹, G. Hamm¹, F. Pamelard¹, S. Madden, and J. Stauber³
¹ImaBiotech, Lille, France, ²Charles River Discovery Research Services, Edinburgh, United Kingdom

Interstitial Pulmonary Fibrosis (IPF) is a chronic and progressive lung disease. It is currently believed that fibrosis is caused by aberrant alveolar epithelial cell activation and repair leading to fibroblastic/myofibroblastic foci, accumulation of extracellular matrix and irreversible destruction of the lung tissue. Combined with classical histological staining, Mass Spectrometry Imaging (MSI) was used to improve the understanding of the Bleomycin IPF rat model and to identify several potential early biomarkers of this pathology. Rats were administered seven doses of Bleomycin delivered to the lungs at 1 mg/kg. Several lung fresh sections were prepared and analyzed by MSI. We describe a process which combines MSI and classical staining approach directly on tissue to follow the distribution of molecules implicated in fibrosis, and allows a better understanding of lung damage and repair. Indeed most of the identified biomarkers were located in extracellular medium and in the plasma membrane, as some upregulated lipids and novel lipids not previously associated with fibrosis. These lipids are known to be involved in cell signaling, chemotaxis or membrane stability, which might be associated with deregulated alveolar epithelial repair and/or fibrosis. Combination of MSI and histological staining provides information regarding molecules distribution and identification in the tissue by studying their co-distribution and by comparing their relative abundance at active sites of fibrosis. Here we identified a number of biomarkers which may be useful diagnostic and/or therapeutic targets and therefore useful tools to help understand the efficacy and safety of novel treatments in IPF.

Poster Number: T-23

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology
NOVEL DIGITAL MEASUREMENT STRATEGY TO ASSESS EXTENT OF THERMAL DAMAGE IN EVALUATION OF ELECTROSURGICAL MEDICAL DEVICES

F. Aeffner¹, C.R. Mahrt¹, E. Hagendorn¹, F.X. Sicotte¹, E.A. Johnson², and G.D. Young¹
¹Flagship Biosciences, Westminster, CO, ²Medtronic, Boulder, CO

Quantification of thermal tissue damage caused by electrosurgical medical devices is a key component of their safety testing and required for regulatory agency approval. Quantification of hematoxylin and eosin (H&E) stained changes in cell and tissue morphology induced by these medical devices can be challenging. The purpose of this work was to develop a digital measurement tool to quantify the depth of the thermal tissue damage in H&E-stained sections of various porcine tissues. An electrosurgical device was used to induce thermal tissue damage in fresh (bench) tissue. Subsequently, these tissues were excised, fixed, processed, sectioned and stained with H&E. Slides were digitized using Aperio XT scanners and viewed with Aperio’s Image Scope software. The upper and lower vertical axes of the thermal damage was manually annotated and verified by a pathologist. A novel algorithm was developed to place an array of parallel measurements that connect perpendicularly to the thermal damage margins previously defined, accurately measuring depth every 25μm (“Two-sided Harp Measurement”). The data collected can be viewed as a spider plot, accurately reflecting regional differences in the extent of the thermal damage. Minimum, maximum and average depth can be evaluated as well. With this work, we demonstrate a novel method to assess the extent of thermal damage in H&E stained sections.

Poster Number: T-24

Section: STP and Industrial and Toxicologic Pathology
Keyword: Bone and Joint

HISTOPATHOLOGIC EVALUATION OF A NOVEL PERCUTANEOUS INTRAMEDULLARY (IM) PHOTODYNAMIC BALLOON STABILIZATION SYSTEM (PBSS) IN SHEEP TIBIA

B.G. Zani¹ and R. Baird²
¹CBSET, Inc., Lexington, MA, ²Stealth BioTherapeutics, Newton, MA

As an alternative to traditional IM fixation, which requires extensive tooling and an inventory of metal ‘pins’, a novel photodynamic balloon stabilization system (PBSS) was developed which requires neither. A histopathologic evaluation of the PBSS in an ovine tibia model was used to assess the safety and feasibility of its use in long-bone fractures. The medullary cavity of 19 sheep tibiae were implanted with a Dacron balloon, subsequently filled with a liquid photo-dynamic monomer which is light-cured in situ. Implanted tibiae were harvested and radiographed at 30, 90, and 180 days post-implant. Cross-sections from the un-deemineralized diaphysis and metaphysis of all treated tibiae were resin-processed and H&E stained. The PBSS-implanted tibia appearance was characterized by consistent contact apposition and anatomical conformation to the diaphyseal endosteal surface of the medullary cavity with no evidence of device-related adverse effects observed locally (e.g., inflammation, fractures,
fibrosis) nor systemically (e.g., pulmonary embolization). Evidence of variable necrosis in the diaphyseal cortical bone was observed; however, the necrosis was interpreted as the type consistent and generally associated with the acute disruption of the endosteal blood supply during pre-implant medullary cavity preparation procedures (i.e., removal of marrow) required for the deployment of IM devices. Regardless, evidence of active remodeling in the areas of necrotic cortical bone, as well as periosteal and/or endosteal bone apposition, were interpreted as indicative of no adverse effects on homeostatic osseous responses. The IM application of a PBSS was interpreted as a biocompatible, and feasible, method for intramedullary fixation.

**Poster Number:** T-25

**Section:** STP and Industrial and Toxicologic Pathology
**Keyword:** Clinical Pathology

**ERYTHROPOIESIS STIMULATING AGENTS: CLINICAL PATHOLOGY EFFECTS IN RATS SECONDARY TO EXTREME ERYTHROCYTOSIS**

L. Cregar
MPI Research, Mattawan, MI

Erythropoietin is a glycoprotein hormone produced by the renal peritubular interstitial cells in response to hypoxia. Erythropoietin stimulates erythroid precursor cells to proliferate and differentiate into mature erythrocytes. Erythropoiesis stimulating agents (ESAs) are synthetic compounds that directly or indirectly mimic the actions of endogenous erythropoietin, and have been developed to treat anemia caused by chronic renal failure, chemotherapy and other etiologies. Several compounds are currently marketed for these purposes, although continual development of novel ESAs is currently underway to improve the safety, potency and half-life of these compounds. In preclinical safety studies with a number of novel ESAs, administration of the ESA to normocytic/hem rats results in the expected pharmacologic effects of increases in reticulocyte counts with resultant erythrocytosis, and corresponding erythroid hyperplasia in the bone marrow, and extramedullary hematopoiesis, primarily in the liver and spleen. These effects can be marked. However, effects on clinical pathology data secondary to extreme erythrocytosis are also commonly noted, particularly at higher dose levels, and may include prolongations in coagulation times (i.e. prothrombin time and APTT), increases in total bilirubin and serum protein concentrations, increases in aspartate aminotransferase activity, and decreases in glucose concentration. Identification of these secondary effects as a result of erythrocytosis and not as off-target compound-related effects is important in the accurate safety assessment and continued development of these compounds.

**Poster Number:** T-26

**Section:** STP and Industrial and Toxicologic Pathology
**Keyword:** Industrial and Toxicologic Pathology

**HISTOPATHOLOGICAL PANCREAS, LIVER, AND GALL BLADDER CHANGES AFTER ORAL ADMINISTRATION OF TGR5 AGONISTS TO DB/DB MICE**
Sanofi–Aventis Deutschland GmbH, R&D Frankfurt, Germany

The bile acid TGR5 membrane receptor is involved in the regulation of glucose and energy homeostasis by GLP1 secretion from enteroendocrine L-cells. TGR5 activation is considered beneficial for prevention and treatment of diabetes and associated diseases. However, limited data on toxic liabilities are published. To identify potential preclinical target organs pancreas, liver and gall bladders were examined microscopically from db/db mice treated orally for 4 weeks at doses of 3 or 30 mg/kg/day with a systemically (RO5527239) or a low systemically (RA450) available TGR5 agonist each. Both TGR5 agonists originate from unrelated chemical series.

Pancreatitiss/fat necrosis consisting of acinar cell degeneration, interstitial edema with inflammatory cell infiltration and focal areas of necrosis in the pancreatic and peripancreatic fat tissue was seen with both TGR5 agonists and at both doses. In gall bladder, an increased size due to diffuse hyperplasia of the epithelium and neutrophilic inflammation was found in animals of both RO5527239 dose groups and of the 30 mg/kg RA450 group. In addition, hyperplasia of the periportal bile ducts, periportal vasculitis/perivasculitis and multifocal hepatocellular necrosis was observed in liver of animals from the RO5527239 dose groups.

**Conclusion:** In db/db mice, systemic and low systemic available TGR5 agonists lead to similar histopathological changes in pancreas, gall bladder and liver suggesting target-related effects.

**Poster Number:** T-27

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Liver and Pancreas

**BIOMARKERS OF HEPATOFIBROSIS FOR SUBACUTE ORAL EXPOSURE TO PROTOTYPIC LIVER TOXINS**

E. Carroll¹, J. Koontz², C. Baer³, and D.L. Ippolito²
¹Army Institute of Public Health, Aberdeen Proving Ground, MD, ²US Army Center for Environmental Health Research, Fort Detrick, MD, ³Exct, Inc., Fort Detrick, MD

More than 80,000 chemicals are in commercial use worldwide; many have not been tested for toxicity. Hepatic metabolism of xenobiotics increases risk of injury when toxic intermediates are formed. Estimating tissue injury with simple, minimally invasive assays is critical to early diagnosis of liver injury. We administered prototypic hepatotoxins (allyl alcohol or carbon tetrachloride (CCL4)) to rats for 5 or 14 days and correlated histopathological assessment of tissue injury with clinical chemistries and serum miR-122, a novel liver-specific biomarker of injury. Livers were processed and scored for inflammation, fibrosis, necrosis, and lipid accumulation. Serum miR-122 concentration was assessed by qPCR. Allyl alcohol produced elevated liver enzymes and bilirubin. More affected animals and more severe lesions were observed as dose increased. CCL4 produced smaller elevations in clinical chemistries than allyl alcohol. The high dose resulted in liver enzymes significantly higher than controls. A dose-dependent increase in lipid accumulation and centrilobular collagen were confirmed. CCL4 (200mg/kg) and allyl alcohol (45mg/kg) were associated with elevated serum miR122 (3.7 +/-0.5 and 4.0+/−2.1 log2-fold
change), respectively, over vehicle controls in rats. MiR-122 values did not correlate with specific pathology, but did correlate slightly with severity of injury. Ongoing studies compare miR122 expression and clinical chemistries with more focused histopathology and genomics investigation to elucidate pathogenesis and mechanisms of fibrosis.

Disclaimer: Research was conducted in compliance with Animal Welfare Act and all Federal requirements. The views expressed are those of the authors and do not constitute endorsement by the US Army.

§ Poster Number: T-28

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

POTENTIAL MODES OF ACTION FOR PERFLUOROOCTANOIC ACID (PFOA)-INDUCED HEPATOCELLULAR HYPERTROPHY IN MICE

E.M. Quist1,2,3, V.A. Chappell1, A.J. Filgo1,4, Y. Wang2, G.E. Kissling5, and S.E. Fenton1
1NTP Laboratory, DNTP, NIEHS, Research Triangle Park, NC, 2Cellular and Molecular Pathology, DNTP, NIEHS, Research Triangle Park, NC, 3Comparative Biomedical Sciences, College of Veterinary Medicine, NCSU, Raleigh, NC, 4Curriculum in Toxicology, UNC, Chapel Hill, NC, 5Division of Intramural Research (DIR), NIEHS, Research Triangle Park, NC

Perfluorooctanoic acid (PFOA) is a widely used industrial surfactant that persists in the environment and has been linked to potentially toxic and carcinogenic effects in animals and people. Pregnant CD-1 mice were orally gavaged with low doses of PFOA (0, 0.01, 0.1, 0.3 and 1 mg/kg PFOA) from gestation days 0 through 17. Livers were collected on post-natal day (PND) 21 and 91 and routinely processed for histological evaluation and transmission electron microscopy (TEM). On PND 21, histopathologic changes in the liver of offspring included hepatocellular hypertrophy and perilobular inflammation that increased in severity by PND 91. Additional significant dose-related changes in PFOA-exposed offspring included decreases in serum cholesterol (HDL, LDL) and lipid biomarkers. At PND 91, liver TEM of CD-1 and similarly exposed Sv/129 mice revealed PFOA-induced cellular damage and mitochondrial proliferation with altered morphologies suggestive of increased or uncontrolled fission and fusion reactions. We conclude that prenatal exposures to low doses of PFOA induce hepatocellular hypertrophy in mice due to mitochondrial proliferation, not peroxisome proliferation. We suggest that the induced mitochondrial changes in these prenatally exposed mice represent altered mitochondrial function that is driving the hypertrophy response, that, when prolonged, may lead to tumor development later in life. Preliminary analyses of hepatic RNA isolated from affected mice, indicate that these prenatal PFOA exposures induce altered genetic expression of key regulators involved in lipid metabolism, cellular proliferation pathways and mitochondrial function.

Poster Number: T-29

Section: STP and Industrial and Toxicologic Pathology
Keyword: Nervous System
APPLICATION OF A COMPACT MAGNETIC RESONANCE IMAGING SYSTEM FOR TOXICOLOGIC PATHOLOGY: EVALUATION OF LITHIUM PILOCARPINE-INDUCED RAT BRAIN LESIONS

Y. Taketa¹, M. Shiotani¹, Y. Tsuru², S. Kotani³, Y. Osada³, T. Fukushima³, A. Inomata¹, J. Sonoda¹, K. Hayakawa², K. Nakano-Ito¹, E. Ohta¹, Y. Seki¹, A. Goto¹, and S. Hosokawa¹
¹Tsukuba Drug Safety, Eisai Co., Ltd.; ²Research Support Dept., Primetech Corp.; ³Neuroscience and General Medicine Product Creation Unit, Eisai Co., Ltd.; ⁴Preclinical Safety Research Laboratories, Sunplanet Co., Ltd.

[Introduction] Magnetic resonance imaging (MRI) is a useful non-invasive tool to detect lesions in clinical medicine. The present study evaluated the availability of a new compact MRI platform for preclinical toxicologic pathology examination of lesions in the rat brain.

[Methods] Male Sprague-Dawley rats were treated once with lithium chloride (127 mg/kg, i.p.) followed by pilocarpine (30 mg/kg, i.p.) to induce brain lesions. One week after dosing, the perfusion fixed brains were collected, analyzed by a compact MRI system (1.05 tesla permanent magnet; Aspect Imaging, Israel), and examined histopathologically.

[Results and Discussion] In the brain of treated rats, MRI imaging demonstrated areas of high T1 and middle to low T2 signals compared to controls were noted in the piriform cortex, lateral thalamic nucleus, posterior paraventricular thalamic nucleus, and posterior hypothalamic nucleus of the cerebrum. In histopathology, well circumscribed foci of neuronal cell degeneration/necrosis accompanied by gliosis were observed in these areas. The close correlation between the presence of altered signal areas and histopathologic lesions demonstrates the usefulness of this current compact MRI system and that the MRI analysis of fixed organs before routine slide preparation could provide useful information for histopathological evaluation especially in complex and functionally heterogeneous organs such as the brain.

Poster Number: T-30

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

PATHOLOGY OF A PEGYLATED THERAPEUTIC PEPTIDE

G. Tyagi¹, A. Braendli-Baiocco⁵, M. Albassam¹, and I. Mikaelian³
¹Roche Innovation Center, New York, New York, USA; ²Roche Innovation Center, Basel, Switzerland; ³Roche, Nutley, New Jersey, USA

Pegylation of biotherapeutics is a widely employed technique to improve their half-life and reduce their immunogenicity. Though polyethylene glycol (PEG) itself is not considered toxic, its accumulation in lysosomes is associated with vacuolation when large amounts of PEG are administered in toxicology studies. A 2.4 kDa peptide with a linear 30 kDa methoxy PEG was developed for Type 2 diabetes. The receptor for the peptide is expressed in several tissues including brain. The compound was administered subcutaneously to rats and cynomolgus moneys in multiple repeat dose toxicity studies up
Immunohistochemically, in some vacuolation CD34 cynomolgus control (Rathke’s pouch remnant), pancreas, eye and urinary bladder. In addition, in the 4-week rat and cynomolgus studies immunolabeling for PEG was observed in brain and spinal cord without evidence of vacuolation. In the 26-week rat and 52-week cynomolgus studies vacuolation and PEG immunolabeling was observed in neurons in brain, spinal cord and dorsal root ganglia which was non-reversible. As some of the nuclei in brain affected by neuronal vacuolation were known to express the receptor, we hypothesized that neuronal uptake of PEG could occur both in a peptide-independent or peptide-dependent manner. No evidence of cell damage was observed in tissues affected by vacuolation. In conclusion treatment with a PEGylated peptide resulted in vacuolation in several tissues; this vacuolation was not associated with cellular damage but was only slowly or not reversible.

**Poster Number:** T-31

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**THE HISTOPATHOLOGICAL EXAMINATION OF URETHANE-INDUCED NEOPLASTIC CHANGES IN Tg.rasH2 MICE**

T. Sano, T. Watanabe, Y. Ishimura, and H. Anayama  
Drug Safety Research Laboratories, Takeda Pharmaceutical Company Limited, Fujisawa, Japan

The purpose of the present study is to define the histopathological characteristics of neoplastic lesions in the lung from the urethane-treated Tg.rasH2 mice (25 mice/sex, 1000 mg/kg/3 times) and vehicle control (50 mice/sex) in the transgenic carcinogenicity study.

Bronchiolar-alveolar adenomas/adenocarcinomas in the lung were observed in all of the urethane-treated animals. In addition, total 10 urethane-treated animals were also shown the hemangiosarcoma in the lung. Bronchiolar-alveolar adenoma was observed in total 3 control animals.

Immunohistochemically, bronchiolar-alveolar adenomas were positive for both alveolar epithelial type II cells (AE2) by surfactant protein C (SP-C) and pulmonary epithelium by thyroid transcription factor 1 in control and the urethane-treated animals. Interestingly, Clara cell 10 (CC10) positive cells were observed in a scattered manner or diffusely in large adenomas/adenocarcinomas, which had also SP-C expressing tumor cells, from the urethane-treated animals. Pulmonary hemangiosarcomas were positive for vascular endothelial markers von Willebrand factor, platelet endothelial cell adhesion molecule and CD34 in the urethane-treated Tg.rasH2 mice.

It is generally believed that the pulmonary tumors originate from either AE2 cells or Clara cells. Recently, the presence of bronchiolar alveolar stem cells (BASC), which can differentiate into AE2 and Clara cells, were reported and considered to have an important role in the lung tumorigenesis. In this study, most of the urethane-induced pulmonary tumors were observed to have an AE2 cell phenotype, but the presence of SP-C and/or CC10 double positive cells, which are similar immunohistochemically consistent with BASC, suggested that some of urethane-induced lung tumors were have arisen from BASC.
INHIBITION OF ISLET AMYLOID POLYPEPTIDE AGGREGATION AND ASSOCIATED CYTOTOXICITY BY NON-STERoidal ANTI-INFLAMMATORY DRUGS

J.S. Fortin and M-O Benoit-Biancamano
Département de Pathologie et de Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute an important pharmacotherapeutic class which, over the past decade, have expanded in application to a panoply of medical conditions. They have been tested for neurodegenerative diseases such as Alzheimer’s to reduce the inflammation and also in the attempt to abrogate amyloid deposition. However, the use of NSAID as aggregation inhibitors has not been extensively studied in pancreatic amyloid deposition. Pancreatic amyloidosis involves the misfolding of islet amyloid polypeptide (IAPP) and contributes to the progression of type 2 diabetes in the human and feline. To ascertain their anti-amyloidogenic activity, several NSAIDs were tested in vitro using fluorometric ThT assays, circular dichroism, photo-induced cross-linking assays and cell viability assays. Celecoxib, diclofenac, indomethacin, meloxicam, niflumic acid, nimesulide, phenylbutazone, piroxicam, sulindac and tenoxicam reduced fibrillization at a molar ratio of 1:10. The circular dichroism spectra of diclofenac, piroxicam and sulindac showed characteristic spectral signatures found in predominantly α-helical structures. The oligomerization of human IAPP was abrogated with diclofenac and sulindac at a molar ratio of 1:5. The cytotoxic effects of pre-incubated human IAPP on cultured INS-1 cells were noticeably reduced in the presence of diclofenac, meloxicam, phenylbutazone, sulindac and tenoxicam at a molar ratio of 1:10. Our results demonstrate that NSAIDs can provide chemistry scaffolds to generate new promising anti-amyloidogenic agents that can be used alone or as a coadjuvant therapy.

CHANGES OF THE OSTEOCLAST INDUCED BY REPEATED ADMINISTRATION OF A CATHEPSIN K INHIBITOR AND BISPHOSPHONATE IN RATS

Central Research Laboratories, Seikagaku Corporation, Tokyo, Japan
Cathepsin K (CatK) inhibitors inhibit bone resorption by mechanisms of action that are distinct from bisphosphonates, and are expected as a new treatment of osteoporosis. In this study, the effects of a CatK inhibitor and bisphosphonate (Alendronate, ALN) on the osteoclast and bone tissue were compared histologically. Female F344 rats were orally administered with a CatK inhibitor at 0, 20, and 500 mg/kg or ALN at 5 mg/kg, once daily for 14 consecutive days, and the femurs and tibiae were examined. In the ALN-treated group, large-sized osteoclasts with larger number of nuclei were frequently seen on the surfaces of the primary spongiosa and some osteoclasts showed apoptosis. In addition, the primary spongiosa was increased in length at the metaphysis. In the CatK inhibitor-treated groups, although no obvious changes were observed by HE staining, stainability of osteoclasts for TRAP (Tartrate-resistant acid phosphatase) was intensely increased on the primary spongiosa at the metaphysis. Ultrastructurally, the vesicles with limiting membranes increased in the cytoplasm of the osteoclast. By electron microscopic cytochemistry, intense TRAP staining was detected in the area of cytoplasm surrounding the increased vesicles and also in some parts of the intra-vesicles. Thus the CatK inhibitor induced different types of changes in the osteoclast and bone tissues as compared with ALN. Since both CatK and TRAP are localized in the intracellular vesicles of osteoclasts and they are functionally related to each other, the CatK inhibitor is thought to induce morphological changes of the vesicles and TRAP contents of the cells.

**Poster Number:** T-34

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Immune System

**HISTOPATHOLOGICAL DIFFERENCE IN LYMPHOID TISSUES OF SD AND F344 RATS IN T-CELL DEPENDENT ANTIBODY RESPONSE ASSAY**

B. Ogawa, Y. Nakanishi, T. Koyama, K. Arima, and M. Sasaki
Taisho Pharmaceutical Co., Ltd. Saitama, Japan

T-cell dependent antibody response (TDAR) assay is a functional test for immunotoxicity, and the immune reactivity of TDAR assay differs among the strains in rats. We performed TDAR assay of Cyclophosphamide (CP), and the histopathological analysis of the various lymphoid tissues in SD and F344 rats was conducted to investigate the difference in the sensitivity of immunotoxicity between the 2 strains.

Six-week-old male SD and F344 rats were treated with CP 0 (control) and 4 mg/kg/day for 28 days orally and keyhole limpet hemocyanin 300 µg/animal on Days 14 and 23 intravenously as an introduced antigen. The concentrations of anti-KLH IgM and IgG in serum were measured. HE staining and immunohistochemistry for the markers of T-cell (CD3) and B-cell (CD45RA) were performed on spleen, thymus and various lymph nodes (mesenteric, submandibular, axillary, popliteal, inguinal, pulmonary and iliac lymph nodes).

In CP-administered rats of both strains, decreased concentrations of anti-KLH IgM and IgG in serum were observed. Histopathological analysis revealed decreased lymphocytes mainly in the B-cell area on HE-stained sections, and decreased CD45RA-positive cells in immunohistochemistry. These changes were more noticeable in F344 rats than SD rats.
The present study demonstrated that the number and activity of the lymphocytes in the lymphoid tissues of F344 rats are low and inactive when compared with those of SD rats, thus suggested F344 rats might be more sensitive for detecting immunotoxicity than SD rats.

**Poster Number:** T-35

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Reproductive System

**THE VALUE OF LONGITUDINAL SECTIONS OF FEMALE REPRODUCTIVE TISSUES FOR NTP STUDIES**

S. Elmore, C. Blystone, and C. Johnson

Extended evaluations of residual female reproductive tissue were performed for six chronic NTP rat bioassays: Tetrabromobisphenol A (TBBPA), Indole-3-Carbinol (I3C), CIMSTAR 3800, Green Tea Extract (GTE), Pentabromodiphenyl oxide mixture (DE-71), and 2,3 Butanedione. Previous sampling methods consisted of one cross section through each uterine horn, sections of both ovaries, and collection of cervix and vagina only when gross lesions were present. For a more thorough evaluation of the female reproductive tract, the residual tissues (stored in 10% neutral buffered formalin) were evaluated, including the remaining uteri, cervices and vaginas. Each tissue was cut in a longitudinal manner and evaluated for neoplastic and nonneoplastic lesions. This new protocol allowed for 10-20 times more uterine tissue for evaluation. The majority of new findings were in the additional uterine tissue, rather than in cervices or vaginas. Additional neoplastic and nonneoplastic lesions were discovered, specifically adenoma, adenocarcinoma, malignant mixed Müllerian tumor (MMMT), stromal polyp, stromal sarcoma, atypical hyperplasia, and cystic endometrial hyperplasia. Evaluation of additional reproductive tissue supported the level of evidence calls of *equivocal* and *some evidence of carcinogenic activity* in Cimstar and I3C respectively and increased confidence in the call for TBBPA (*clear evidence*). Moreover, a statistically significant increase in cystic endometrial hyperplasia was noted in the original review of TBBPA, but was not present in the extended review. For the remaining chemicals, the lack of a statistically significant increase in uterine tumors upon examination of the residual tissue supported the call of *no evidence of carcinogenic activity* from the original review.

**Poster Number:** T-36

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Nervous System

**PRECLINICAL EFFICACY TESTING OF THE MITOCHONDRIA-TARGETED ANTIOXIDANT MITOAPOCYNIN IN THE TRANSGENIC MITOPARK MOUSE MODEL OF CHRONIC DOPAMINERGIC NEURODEGENERATION**

M.R. Langley, A. Ghosh, M. Ay, B. Bennett, H. Jin, V. Anantharam, B. Kalyanaraman, A. Kanthasamy, and A. G. Kanthasamy
Advances in drug discovery for neurodegenerative diseases, including Parkinson’s disease (PD), have been hampered by the lack of chronic animal models that recapitulate the slow and progressive neurodegeneration. A genetic mouse model of mitochondrial dysfunction known as MitoPark was recently developed by selectively knocking out the mitochondrial transcription factor Tfam in dopaminergic neurons. Recently, we developed a novel class of drugs targeting mitochondria to effectively dampen the major pathophysiological processes, mitochondrial dysfunction and oxidative damage. In this study, we evaluated the neuroprotective efficacy of a mitochondria-targeted apocynin derivative, mito-apocynin, in this model. MitoPark mice progressively exhibit mild to severe motor deficits over 12-24wks, along with gradual loss of striatal dopamine and nigral dopaminergic neurons. However, MitoPark mice orally administered 10 mg/kg mito-apocynin (13-24wks, 3x/wk), significantly improved their coordination, stability and spontaneous locomotor activity relative to vehicle-treated MitoPark mice, as measured by RotaRod and open-field tests. Mito-apocynin also recovered the loss of dopamine and dopaminergic neurons. Electron paramagnetic resonance (EPR) spectroscopy revealed that fractional intensities of reduced iron-sulfur clusters and inactive forms of aconitase were significantly higher in MitoPark brains compared to those from age-matched littermate controls, while mito-apocynin administration decreased these levels. Furthermore, the higher brain levels of oxidative damage markers, 4-HNE and iNOS in MitoPark mice relative to controls were significantly reduced by mito-apocynin. Collectively, our data reveal that mito-apocynin mitigates oxidative and nitritative damage, rescues behavioral deficits and dopamine depletion, and protects against neurodegeneration in the MitoPark mouse model of PD (NIH grants NS074443 and ES10586).

**Poster Number:** T-37

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Industrial and Toxicologic Pathology

**GENETIC AND RAT TOXICITY STUDIES OF CYCLODEXTRIN GLUCANOTRANSFERASE**

S. Hayashi, J. Davis, C. Hobbs, C. Swartz, M. Boyle, L. Recio, and R. Maronpot

Introduction: Microbiologically derived cycloextrin glucanotransferase (CGTase) is used commercially as a processing agent in manufacture of food, pharmaceuticals, and cosmetics. Its toxic potential was evaluated in anticipation of use in the production of alpha glycosyl isoquercitrin, a water-soluble form of quercetin.

Methods: Following OECD guidelines, CGTase, produced by *Bacillus pseudocalphilus* DK-1139, was evaluated in a genotoxicity battery consisting of a bacterial reverse mutation assay, in vitro and in vivo micronucleus (MN) assays, and a comet assay using B6C3F1 male and female mouse tissues. These same genotoxicity assays were also conducted for sodium sulfate, a contaminant of CGTase preparation. In a 90-day Sprague Dawley rat toxicity study, CGTase was administered by gavage in water at daily doses of 0, 250, 500, and 1000 mg/kg/day.
Results: CGTase did not induce mutations with or without metabolic activation in the bacterial reverse mutation assay. Formation of micronuclei was not induced in either in vitro or in vivo MN assays with or without metabolic activation. No induction of DNA damage was detected in male or female mouse liver, stomach, or duodenum in the comet assay. Sodium sulfate also tested negative in these same genotoxicity assays. In the 90-day repeated dose rat study there were no treatment-related adverse clinical or pathological findings.

Conclusion: The genotoxicity assays and repeated dose toxicity study support the safe use of CGTase in production of alpha glycosyl isoquercitrin.

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**Poster Number:** T-38

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Immune System

**FLOW CYTOMETRY AND IMMUNOPHENOTYPING IN DRUG DEVELOPMENT**

T. Papenfuss, J. Figueiredo, R. Tadagavadi, N. Markori, and G. Parker  
WIL Research Laboratories, Ashland, OH

With an increasing number and array of biopharmaceuticals, it is critical that laboratories engaged in product development can adequately evaluate effects on the immune system. Flow cytometry is a rapid and robust means to evaluate immunotoxicologic, immunopathological and immunomodulatory effects which can be applied throughout drug development pipeline to help define mechanisms of action, pharmacokinetics, lead optimization, cell toxicity, immunomodulation and biomarker monitoring. Immunophenotyping can evaluate the impact of biopharmaceuticals on the proportion of immune cell populations that cannot be ascertained from routine hematology or histology data. Blood immunophenotyping is commonly employed in various species to assess the levels of T, B and NK cells and can be used to evaluate rare populations (e.g. myeloid) within the blood. Tissue-based immunophenotyping of immune and non-immune (e.g. tumor) tissues provide organ-specific information. Using CD4, CD8, CD45RA+ and CD161a+ markers, we identified helper and cytotoxic T, NK, NK-T and B cells within the rat splenocytes. Using CD3, CD11b/c, B220, CD4, CD8 and T cell receptor markers, we identified specific thymic populations (i.e. 22% CD4+CD8-, 8% CD4-CD8+, 60% CD4+CD8+ and 9% CD4-CD8- populations which can be used to evaluate thymic development. Enhanced and functional immunophenotyping are new areas of flow cytometry which can determine subtle effects of immunomodulatory compounds and can be used in conjunction with routine immunophenotyping, histopathology, immunohistochemistry and various other techniques. With the increasing diversity of immunomodulatory biopharmaceuticals, it will be necessary and expected to provide a robust evaluation of immune alterations for translating animal data to predict possible human risk.

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**Poster Number:** T-39

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Urinary System
COMPARISON OF RENAL AMYLOID AND HYALINE GLOMERULOPATHY IN B6C3F1 MICE: AN NTP RETROSPECTIVE STUDY

J.S. Hoane\(^1\), C.L. Johnson\(^1\), J.P. Morrison\(^2\), and S.A. Elmore\(^2\)
\(^1\) Charles River Laboratories, Pathology Associates, Durham, NC, \(^2\) National Toxicology Program (NTP), National Institute of Health and Environmental Sciences, Research Triangle Park, NC

Due to potential misdiagnosis of hyaline glomerulopathy (HG) for glomerular amyloidosis, a retrospective study of B6C3F1 mouse kidneys from the National Toxicology Program (NTP) archives was undertaken to determine if HG had occurred in prior NTP studies and, if so, whether these two glomerular lesions could be routinely discriminated. Kidneys from amyloid and HG positive control mice, and 41 potential HG mice previously diagnosed with “renal only” amyloidosis, were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Congo red (CR) and Masson’s trichrome and evaluated by light microscopy. Ultraviolet (UV) evaluation of H&E and CR stained slides was also performed. All 41 potential HG mice had glomerular deposits histochemically consistent with HG and inconsistent with amyloid. The deposits were similar to HG positive controls but not amyloid positive controls as the deposits were all CR negative, bright pink with PAS staining, and autofluorescent in H&E stained sections with UV illumination. Four of the 41 potential HG mice were randomly selected for transmission electron microscopy which confirmed that the glomerular deposits in these mice were consistent with HG and not amyloid. Our findings indicate that HG is a spontaneous lesion in B6C3F1 mice of low occurrence, may be misdiagnosed as amyloidosis, and is more likely to cause glomerular deposits than amyloid in mice without evidence of deposits in other tissues such as liver, spleen or intestine. Also, HG can be distinguished from amyloid on H&E evaluation; however, the distinction is improved with PAS or CR staining, and/or UV evaluation.

**Poster Number:** T-40

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**IN VIVO ASSESSMENT OF RITUXIMAB-EU (MABTHERA\(^\circ\)) AND PF-05280586 IN CYNOMOLGUS MONKEYS**

A. Ryan\(^1\), S. Sokolowski\(^1\), M. Collinge\(^1\), A. Shen\(^1\), J. Arrington\(^1\), N. Shirai\(^1\), T. Cummings\(^1\), S. Ploch\(^2\), S. Stephenson\(^2\), N. Tripath\(^2\), S. Hurst\(^2\), G. Finch\(^1\), and M. Leach\(^1\)

\(^1\)Pfizer, Inc., \(^2\)Covance Laboratories, Inc.

PF-05280586 is a proposed biosimilar to the marketed rituximab products Rituxan\(^\circ\) and MabThera\(^\circ\), which are anti-CD20 monoclonal antibody agents that deplete B cells and are used in the treatment of rheumatoid arthritis and B cell lymphoma. The pharmacodynamic effects of PF-05280586 and rituximab-EU (MabThera\(^\circ\)) were compared in sexually-mature cynomolgus monkeys in single-dose pharmacokinetic and repeat-dose toxicity studies, both of which had a 13-week observation period to assess B cell repletion. Peripheral blood lymphocytes were evaluated by flow cytometry to characterize peripheral B cell depletion/repletion using a panel of markers. B cells were evaluated microscopically in spleen, axillary and mesenteric lymph nodes using CD20 immunohistochemistry (repeat-dose only). The magnitude and time course of B cell depletion and repletion were similar
between PF-05280586 and rituximab-EU and were consistent with the expected pharmacology of anti-CD-20 monoclonal antibodies and the nonclinical data previously reported by the innovator. PF-05280586 is currently in Phase 3 clinical studies.

**Poster Number:** T-41

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**COMPARATIVE PHARMACOKINETICS AND SAFETY OF TRASTUZUMAB-US AND TRASTUZUMAB-EU AND THE POTENTIAL BIOSIMILAR PF-05280014 IN CD-1 MICE**

A. Ryan¹, S. Hurst¹, J. McNally¹, L. Lorello¹, P. Schmidt², G. Finch³, S. Ploch³, J. Fohey³, and M. Leach²  
¹Pharmacokinetics, Dynamics and Metabolism, Pfizer, Inc., ²Drug Safety Research and Development, Pfizer, Inc., ³Covance Laboratories, Inc.

PF-05280014 is a potential biosimilar to the marketed trastuzumab product Herceptin®, an anti-Her 2 monoclonal antibody used in the treatment of Her-2 over-expressing breast and gastric cancer. The pharmacokinetic parameters of PF-05280014 and Herceptin® approved in the US and EU (trastuzumab-US and trastuzumab-EU, respectively) were compared in CD-1 male mice following a single-dose bolus intravenous injection of 0, 1, 10 or 100 mg/kg. The potential toxicity of PF-05280014 was evaluated following twice weekly injections of 0, 10 or 100 mg/kg (total of 5 doses) in CD-1 mice of both sexes. PF-05280014 was well-tolerated in both studies; all animals survived to their scheduled termination, with comparable body weight gains and clinical observations across all groups (single-dose study). The pharmacokinetic parameters and overall anti-drug antibody incidence were similar among PF-05280014, trastuzumab-US and trastuzumab-EU. There were no test-article related findings in the repeat-dose toxicity study. The results of these 2 nonclinical studies supported the further development of PF-05280014, now in Phase 3 studies.

**Poster Number:** T-42

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Eye and Ear

**LENS CAPSULE PERFORATION WITHOUT INFLAMMATION IN FOUR RABBITS FROM INTRAVITREAL INJECTION STUDIES**

C. Farman, E. Thackaberry, C. Schuetz, F. Lorget, and V. Bantseev

The traditional teaching is that whenever the lens capsule is compromised, ocular inflammation always occurs, due to the release of previously sequestered lens protein. Three different single dose intravitreal injection/implantation studies of four different test materials were performed in New Zealand White rabbits. The test materials included polymer microspheres, polymer rods, a solvent and a hydrogel. The studies ranged from 4 to 6 weeks in length. On Day 1, intravitreal injection/implantation (in the case of
the rods) procedures were performed following aseptic preparation of the eye. Ophthalmic examinations were performed by board certified veterinary ophthalmologists periodically throughout the course of each study, and included indirect ophthalmoscopy and slit lamp examination. None of the affected animals received corticosteroids or other immunomodulatory agents during the course of the studies. Four rabbits had perforation of the posterior lens capsule during the in-life phase of the study, visible on clinical ophthalmic exam as lens capsule alterations described as “lens hits”, and/or incipient posterior cataracts. In all cases it was either known or presumed that the lens capsule had been compromised during the injection/implantation procedure on Day 1. Histologically, there was no evidence of inflammation in association with extruded lens protein material in any of the affected eyes. These results indicate that iatrogenic damage to the lens capsule during aseptically-performed intravitreal injections/implantations does not appear to induce inflammation in rabbits. Introduction of certain antigenic and/or infectious material may be necessary in order for inflammation to occur in association with lens capsule perforation.

Poster Number: T-43

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

CADMIUM INDUCED NEPHROTOXICITY AND GONADOTOXICITY IN FEMALE JAPANESE QUAIL (COTURNIX JAPONICA) AND AMELIORATION BY SILYMARIN

S.L. Butt1, M.Z. Khan2, A. Khan2, M.K. Saleemi2, and M.W. Tahir2
1Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA,
2Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

Cadmium (Cd) is a toxic environmental pollutant, which can accumulate to harmful concentration in body tissues through food and water source exposure. Silmyrin (SL), is a bioactive extract of milk thistle plant (Silybum marianum) and have protective effects against drug induced toxicities. Present study was designed to evaluate potential ameliorative effects of SL against Cd-induced renal and gonadotoxic effects in female Japanese quail. One hundred and twenty quail chicks of four weeks of age were assigned into six groups and given basal feed, Cd1 (as 150 mg CdCl2 Kg⁻¹ feed), Cd2 (as 300 CdCl2 mg Kg⁻¹ feed) and SL (as 250 mg Kg⁻¹ feed) alone and in combination for 60 days. Relative organ weights, serum biochemical analysis, gross and histopathological alterations in kidney and oviduct were noted. Cd1 and Cd2 fed chickens had elevated blood urea nitrogen, creatinine, and serum activity of AST and ALT. Birds from these groups had subjectively enlarged kidneys and atrophied oviducts, which was supported by increased mean kidney weights and decreased mean oviduct weights (P<.05). In the oviduct of Cd-fed chickens mucosal folds were short, mucus-producing tubular glands were collapsed, and nuclei were often degenerated. The kidneys of Cd-fed birds were disrupted by hemorrhages, tubular proteinaceous renal casts and interstitial infiltrates of mononuclear cells. Co-treatment with SL and Cd reduced the Cd-induced gross and histopathological changes in kidney and oviduct along with urea and creatinine levels suggesting its protective role against Cd-induced renal and gonadotoxic effects.

Poster Number: T-44
TECHNICAL CONSIDERATIONS FOR SEMI-QUANTITATIVE THYROID HISTOMORPHOMETRY EVALUATION AND INTERPRETATION


Regulatory guidelines suggest evaluation of endocrine disruptor potential of synthetic chemicals on thyroid homeostasis. Although, the OPPTS (890:1500 and 890:1450) guidelines have outlined certain technical considerations to achieve systematic evaluation of thyroid glands (TG) and TG-related endpoints, there is no clear guidance on the semi-quantitative TG histomorphometry and its interpretation, which is largely subjective. Our objective is to highlight key technical considerations for the semi-quantitative TG histomorphometry and its interpretation. We present technical points based on a recently completed F2-Extended Two-Gen Repro Toxicity Study in the Wistar Rat. In that study, a total of 3040 TG sections (1952 from adult rats and 1088 from PND 23-45 pups) were evaluated and histopathology data was corroborated with TG-hormone data. The key points include, TG collection techniques, weighing, use of dissecting microscopes, microdissection instruments and histology techniques. We also emphasize the importance of setting the objective criteria for microscopic evaluation of follicular height and colloid area (scoring on a scale of 1 to 5) and selection of statistical tools for corroborating TG-histomorphometry and hormone data. We also highlight the importance of standardizing the microscopic evaluation criteria within a laboratory to have a robust historical control data and also across laboratories to avoid variability in data evaluation. Our evaluations concluded that, there should be uniform grading criteria for males vs females of same age group, to establish a range for adults vs pups and to examine the entire section and averaging the grade across all regions.

INTRAMUSCULAR INJECTIONS IN RABBITS: IMPROVING TISSUE COLLECTION AND ASSESSING MULTIPLE TISSUE SECTIONS FOR ACCURATE HISTOPATHOLOGIC EVALUATION

K. Nelson, A. Doan, M. Ashley, and C. Hollinger
MPI Research, Mattawan, MI

Delivering complete and consistent histopathologic sections of intramuscular (IM) injection sites in rabbits within a high production laboratory environment can be challenging. For many test articles, the primary histopathological findings may be limited to the immediate injection site region, and factors such as the loose attachment of rabbit skin to the underlying muscle and acquisition of only single sections contribute to unacceptable margins of error for injection site identification, collection, and
evaluation. A series of IM injections in common locations (lumbar epaxial, quadriceps, biceps femoris, and gastrocnemius muscles) used dye injections on euthanized rabbits to improve postmortem site localization and collection IM dye location was assessed macroscopically following fixation. Shifting of skin position relative to underlying muscle following collection and fixation was dramatic, but was ameliorated by systematic apposition and immobilization of the tissues using surgical staples. Accuracy of IM injection site collection was also improved by increased training in anatomy-based site localization and modified dosing methods by animal dosing technicians; and increased focus on anatomy-based site localization by surgeons. Evaluation of rabbit IM injection toxicology studies assessed the success of changes in postmortem tissue handling and the utility of standard examination of multiple tissue sections from each injection site. Systematic, specialized injection site collection, coupled with microscopic evaluation of multiple sections, provides for accurate injection site identification and recovery rates of 80-100% at common IM injection sites, where single section evaluation is less precise. These postmortem techniques allow consistent and accurate evaluation of IM injection sites.

**Poster Number:** T-46

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Eye and Ear

**FLUOROQUINOLONE-INDUCED RETINAL DEGENERATION IN A DOMESTIC SHORTHAIR FELINE**

T. Peterson¹ ², S. Merchant³, J. Taboada³, E. Storey³, and R. Bauer¹ ²  
¹Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA,  
²Louisiana Animal Disease Diagnostic Laboratory, Baton Rouge, LA, ³Department of Veterinary Clinical Sciences, LSU School of Veterinary Medicine, Baton Rouge, LA

A 17-year-old, female spayed, domestic shorthair feline was submitted for postmortem examination with a history of long term blindness (at 3 years of age) and more recent history of non-related progressive weight-loss and left sided circling (at 14 years of age). At 2 years of age the cat had been diagnosed with cutaneous Actinomycetes sp. and secondary coagulase-positive Staphylococcus sp. infection. The skin lesions were treated with long term antibiotic therapy, including intermittent Enrofloxacin over a one year period. Gross findings consisted of multifocal tan masses in the left kidney, lungs and heart with regional lymphadenomegaly. Histologic evaluation revealed poorly differentiated renal carcinoma with metastases to heart, brain, lung and lymph nodes. These findings explain the more recent signs of the cat. In the eyes there was bilateral, severe degeneration of the retina especially involving the outer layers. There was complete loss of outer nuclear and plexiform layers, multifocal loss of retinal pigmented epithelium and complete loss of photoreceptors. Enrofloxacin-induced retinal degeneration is a well-accepted entity and primarily involves degeneration of the outermost retinal layers. This cat received Enrofloxacin therapy prior to the knowledge of retinal toxicity caused by fluorquinolones. The accepted pathogenesis is a mutation in cats of ABCG2, a transmembrane transporter, in which dysfunction allows the drug to enter the retina and cause photosensitivity of the outer retinal structures. This case provides an interesting example of Enrofloxacin toxicity in a young (3 y/o at time of antibiotic administration), systemically healthy cat leading to retinal degeneration.

**Poster Number:** T-47
CHARACTERIZATION OF RAT MALIGNANT FIBROUS HISTIOCYTOMA (MFH)-DERIVED CLONED CELL LINES (MT-8 AND MT-9) BASED ON THE TUMOR STEM CELL THEORY


MFHs show a storiform growth pattern consisting of fibroblastic, histiocytic and undifferentiated cells with possible multipotency. The histogenesis remains undetermined. We analyzed two cloned cell lines (MT-8 and MT-9) isolated from a spontaneous rat MFH. MT-8 had less developed organelles and the induced tumors represented characteristics of sarcoma NOS, whereas MT-9 cells had well-developed organelles and the tumors showed a storiform growth pattern. MT-8 and MT-9 tumors were immunopositive for vimentin, and stem cell markers (nestin, CD34, CD90, A3) with greater reactivity in MT-9 cells; their tumor cells did not react to epithelial, myogenic and neurogenic markers. The microarray analyses revealed that gene profiles relating to “cell differentiation” were more activated in MT-9 than MT-8 tumors, whereas those involved in “cell cycle” were greater in MT-8 than MT-9 tumors. Additionally, gene profiles involved in “cell differentiation” were much greater in tumors than in cultures of MT-8 and MT-9. These findings indicate that MT-8 cells are poorly differentiated mesenchymal stem cells, whereas MT-9 cells have more differentiated stem cell nature with greater multipotential differentiation. In MFHs, collectively, MT-8 and MT-9 cells are regarded as “tumor stem cells” and “tumor precursors” in the stem cell lineage, respectively, according to the concept of “tumor stem cell theory.”

EX VIVO COMPACT MAGNETIC RESONANCE IMAGING (MRI) OF THE BRAIN WITH HISTOPATHOLOGY VALIDATION IN A RAT MODEL OF HUNTINGTON DISEASE

Y. Schifferbauer1, E. Vezzali2, E. Weber2, R. Pietrek2, M. Steiner2, and A. Nyska3
1Aspect Imaging, Shoham, Israel, 2Actelion Pharmaceuticals Ltd., Allschwil, Switzerland, 3Consultant in Toxicologic Pathology, Timrat and Tel Aviv University, Israel

Introduction: Ex-vivo Magnetic Resonance Imaging (MRI) of fixed biological samples allows the thorough examination of the entire specimen using multiple digital slices, leaving the specimen intact for subsequent investigations, like histopathology. We tested MRI in a rat model of Huntington's disease and validated the results by histopathology.
Materials and Methods: The classical neurotoxin 3-nitropropionic acid (3NP) was administered at 55 mg/kg/day for 8 days to 3 Lewis rats via osmotic mini-pump (i.p.) in order to induce lesions in the nucleus caudatus and putamen of the brain corresponding to the striatum in humans, the structure where the Huntington’s disease associated lesions are prominent. Three rats served as untreated controls. Formalin perfusion was applied at necropsy. Sixty-four transverse digital sections of each brain were acquired by a compact MRI system (Aspect Imaging). In addition, transverse sections stained with Hematoxylin-Eosin and Cresyl violet-Luxol Fast Blue were prepared for histopathologic examination. Results: Ex-vivo MRI targeted the location and the size of neuropathological lesions with good precision. Histopathologically, the lesions were described as neurodegeneration, characterized by hemorrhage and spongiosis in the nucleus caudatus and putamen. Some unclear MRI signals were identified as sampling artifacts.

Conclusion: The ex-vivo MRI was successful in localizing the 3NP-induced neurotoxic lesions, allowing the quantification of the damage and the correlation to histopathology. The MRI technique is able to cover the whole rat brain and, being non-invasive, can be used for time course observations of the neurotoxic changes.

Poster Number: T-49

Section: STP and Industrial and Toxicologic Pathology
Keyword: Primates

EXAMPLES OF PATHOLOGY RELATED TO OPPORTUNISTIC INFECTIONS CONSECUTIVE TO IMMUNOSUPPRESSIVE OR IMMUNOMODULATING AGENTS IN MACAQUES

C. Sobry, F. Gervais, R. Forster, and B. Palate
CiToxLAB, Evreux, France

Introduction: Many novel immunosuppressive or immunomodulating drugs are in preclinical development for organ transplantation, autoimmune diseases, inflammatory diseases or cancer. The non-human primate (NHP) is often the non-rodent species of choice for preclinical studies with these drugs. Objective: We intend to illustrate selected pathology related to opportunistic infections in macaques treated with compounds belonging to diverse classes of small and large molecules and targeting different pathways of immune activation. Experimental design: A total of 106 monkeys were treated for subacute or chronic duration in 6 studies with 4 distinct compounds. In addition to routine evaluation with hematoxylin-eosin staining, immunohistochemistry, in situ hybridization and/or PCR were used to characterize some causative viral pathogens. Methods: A retrospective evaluation of histopathological results and a short literature review were performed, and were illustrated by microphotographs of representative lesions. Results: Systemic cytomegalovirus infections were observed with a high incidence after one month and 13 weeks in two studies with one immunosuppressive drug. Four lymphomas related to lymphocryptovirus infections were found after at least 10 weeks of treatment in 2 studies with one immunosuppressive or one immunomodulating agent. Five cases of septicemia occurred from the fourth week of treatment in 2 studies with 2 distinct immunosuppressive drugs. Gastrointestinal opportunistic infections were seen in all studies. Conclusion: Lesions induced in NHP by common opportunistic agents including latent viruses may confound the
interpretation of studies with immunosuppressive/immunomodulating drugs. **Impact statement:** Opportunistic infections in monkeys are also indicative of the risk of infections in humans treated with those drugs.

**Poster Number:** T-50

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Eye and Ear

**KEEPING AN EYE ON MOLECULAR IMAGING: ASSESSMENT OF DRUG TOXICITY IN SMALL OCULAR STRUCTURE USING MASS SPECTROMETRY IMAGING**

G. Hamm¹, F. Brignole-Baudouin², N. Desbenoit²,³, A. Brunelle⁴, J. Stauber¹, and C. Baudouin²,⁴

¹ImaBiotech, Loos, France, ²Institut de la Vision, Paris, France, ³ICSN, Gif-sur-Yvette, France, ⁴Centre Hospitalier National d'Ophthalmologie des Quinze-Vingts, Paris, France

Mass spectrometry Imaging (MSI) applications to ophthalmic drug discovery have recently gained growing interest especially for preclinical studies in pharmacology or toxicology. In our study, MSI was applied to assess the distribution of Benzalkonium chloride (BAK) compound (antiglaucoma eye drops preservative) in specific areas of the eye after instillation in animal model tissues. They have been reported to cause ocular surface disorders with tear film alteration, eye irritation and to promote dry eye. The distribution of BAK compound was investigated in small specific histological regions of the eye in order to estimate efficiency of action or adverse effects of the treatment. High spatial resolution images were performed at cells level (30 µm). Local Drug concentration differences were observed according to histological area and position on the eye section (anterior, posterior, temporal or nasal side). MSI and IHC results were put side by side to correlate inflammatory areas (degradation of CSE or apoptosis phenomena within cornea/conjunctiva region) with BAK localization. Moreover, a high accumulation of BAKs were observed at the sclerocorneal junction and near trabecular meshwork involved in aqueous humor outflow. MSI offers new insight in ocular therapeutic/pharmaceutical research, especially to give a better understanding of the drug candidate migration from the front to the back of the eye to assist drug efficiency or toxicity studies for specific tissue targeting eye diseases.

**Poster Number:** T-51

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Industrial and Toxicologic Pathology

**A GUIDE TO THE COLLECTION AND MORPHOLOGICAL ASSESSMENT OF ADIPOSE TISSUE IN THE NORMAL, DIABETIC, AND OBESE MOUSE**

J. Ooi, K. Kang, and K. Keane

Novo Nordisk, Beijing, China
Introduction: Understanding the normal biology and pathology of adipose tissue has become a critical need due to dramatic increase in obesity-related diseases. Furthermore, there is a growing awareness of individual differences in the various adipose depots which appear to influence the pathogenesis of metabolic diseases. However, detailed descriptions of how to collect and analyze the three subtypes of fat (Brown, Beige, and White) are lacking.

Methods/Result: We compare the normal (C57/Bl6) to the spontaneously diabetic (db/db) and diet-induced obese (DIO) male mice to produce high-resolution anatomical images with a standardized dissection. This method produces rigorous, depot specific data for the intrascapular, subcutaneous, inguinal/gonadal, and epididymal adipose tissue. Data includes organ weight, lean/fat ratio, adipose morphology, and assessment of adipose biology via immunohistochemistry of fat browning markers in combination with whole-slide image analysis.

Conclusion: We observe that there are anatomical differences of both quantity and composition in the various adipose depots between the spontaneous and diet-induced mice that may influence the pathogenesis of metabolic disease. White adipocytes are found in many anatomical locations, including the subcutaneous, inguinal/gonadal, and epididymal fat pads, while brown adipocytes are found predominantly in the intrascapular pads, and beige adipocytes are present in the inguinal/gonadal region.

Poster Number: T-52

Section: STP and Industrial and Toxicologic Pathology
Keyword: Urinary System

ACUTE RENAL FAILURE AND PAPILLARY CREST NECROSIS IN A YOUNG ADULT CAT FOLLOWING INCIDENTAL CONTACT WITH A COMPOUNDED TOPICAL MEDICATION CONTAINING FLURBIPROFEN

M.T. O’Brien, S.C. Griffin, and B.F. Porter
Departments of Veterinary Pathobiology and Small Animal Clinical Sciences, Texas A&M University, College Station, USA

A one-year-old, intact female, Siamese mixed-breed cat was presented with a one-day history of muscle tremors, shaking, lethargy, and defecating outside the litter box. Clinical evaluation revealed severe dehydration and acute renal failure, and due to the poor prognosis, the patient was euthanized. Relevant necropsy findings included bilateral renal papillary crest necrosis, ulceration of the pyloric antrum mucosa, and melena throughout the gastrointestinal tract. Histologically, renal changes included papillary crest necrosis and multifocal, mild, chronic lymphoplasmacytic and eosinophilic interstitial nephritis. In the context of necropsy findings, follow up questioning of the owner revealed their use of a compounded topical medication containing 10% flurbiprofen. The owner observed that the medication would commonly leave a residue on skin and clothing. Additionally, they reported that the cat would often lie on the owner’s neck, which was a site of topical product use. Papillary crest necrosis is an uncommon but distinctive lesion and can occur secondary to many causes of renal medullary ischemia, including non-steroidal anti-inflammatory drugs (NSAIDs), amyloidosis, pyelitis, lower urinary tract obstruction, and pelvic calculi, among others. Given the young age of the cat, absence of other findings,
and relevant history, acute renal failure and papillary crest necrosis are presumed to be secondary to incidental ingestion of the owner’s topical medication containing an NSAID. This case highlights a possible risk to pets that come into contact with topical NSAID-containing medications used by their owners.

§ Poster Number: T-53

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

SPONTANEOUS BACKGROUND FINDINGS IN ALBINO HARTLEY GUINEA PIGS AT MPI RESEARCH

D.B. Snider¹, P.I. Cole², and R.L. Tapp²
¹Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA, ²MPI Research, Mattawan, MI, USA

Guinea pigs are increasingly promoted for use in ototoxicity studies in addition to models of certain human diseases. A retrospective study of histologic findings was performed to document spontaneous background findings in albino Hartley guinea pigs at MPI Research. Findings were tabulated for 85 animals (26 males and 59 females) less than six months of age. Findings were most prevalent in the kidneys (85.9%), liver (50.6%), heart (41.2%), testes (41.18%), and lungs (30.8%). Mineralization (74.1%) and tubular basophilia (48.2%) were the two most prevalent findings in the kidneys. Mononuclear infiltrate (47.0%) and myofiber vacuolation (16.5%) were the most prevalent findings in the liver and heart, respectively. Seminiferous tubule degeneration/atrophy (30.7%) was the most prevalent finding of the testes. Hemorrhage (11.7%) was the most prevalent finding of the lungs. Male guinea pigs had increased incidence of findings compared to females in the lacrimal glands (13-fold), mesenteric lymph node (3-fold), salivary mandibular glands (2-fold), lung (1.5-fold), and skeletal muscle (1.5-fold). Female guinea pigs had approximately 2-fold increases in findings of the thymus and larynx compared to males. Some previously described background findings of guinea pigs may be lacking as these studies utilized juvenile guinea pigs. Documenting frequency of spontaneously occurring findings will aid in discernment of compound-related effects in subsequent toxicity studies. The prevalence of several findings varies with gender and differences are potentially related to housing, behavior, or institutional factors. This study provides a review of background findings in albino Hartley guinea pigs at a large preclinical CRO.

Poster Number: T-54

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

NORMAL PHYSIOLOGICAL AND PATHOLOGICAL VALUES FOR THE SINCLAIR MINIATURE SWINE

C. Shoemake, D. Brocksmith, A. Stricker-Krongrad, and G. Bouchard
The miniature swine are increasingly recognized as a non-rodent model in regulatory toxicology. The Sinclair miniature swine (SMS) is the oldest strain of miniature swine developed for research and one of the smallest breeds. The similarities between the cardiovascular, renal, and digestive systems make the miniature swine a suitable animal to model the human counterpart. The miniature swine are also the most recognized species for dermal toxicology. The SMS has other attractive traits that make them a good substitute to model humans. They are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia if fed a high fat diet. All routes of compound administration can be used with miniature swine. The SMS should be considered as one of the non-rodent species in systemic toxicity testing. In an effort to generate a database on baseline information about the normal physiological status of the Sinclair miniature swine, we reported physiological data from normal intact and naïve three-month-old Sinclair miniature swine of both genders. The normal physiological data gathered includes growth parameters, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights.

**Poster Number:** T-55

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**NORMAL PHYSIOLOGICAL RANGES FOR HANFORD MINIATURE SWINE**

C. Shoemake, D. Brocksmith, A. Stricker-Krongrad, and G. Bouchard

The miniature swine have been increasingly recognized as a non-rodent model in regulatory toxicity. Members of the FDA have even published on the use of miniature swine as an alternative to canine and non-human primates in regulatory toxicity. The similarities between the cardiovascular, renal, and digestive systems make the miniature swine a suitable animal to model the human counterpart. The miniature swine are also the most recognized species for dermal toxicology. The Hanford miniature swine (HMS) has other attractive traits that make them a good substitute to model humans. They are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia if fed a high fat diet. With the advent of new techniques, all routes of compound administration can be used with miniature swine. The HMS should be considered as one of the non-rodent species in toxicity testing. In an effort to generate a database on baseline information about the normal physiological status of the Hanford miniature swine, we report expanded and updated physiological data from normal intact and naïve juvenile and young adult miniature swine of both genders. The normal physiological data gathered includes growth parameters, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights.

**Poster Number:** T-56

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**A RETROSPECTIVE ANALYSIS OF HOUSING CONDITION-RELATED EFFECTS IN TWO-YEAR RAT CARCINOGENICITY STUDIES**
Rodent toxicology studies have historically been performed in wire-bottom cages. However, both the National Research Council (NRC) and Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) International, recommend housing rats in solid-bottom cages with bedding to prevent development of foot lesions and/or stress responses. A retrospective analysis of Sprague-Dawley rat carcinogenicity study data (n=25 control groups) compared the effects of individual vs. pair housing and wire-bottom vs. solid-bottom housing on body weight, food consumption, survival, incidence of foot lesions and tumor rate. Survival was analyzed by the Cox proportional hazards model; body weight was analyzed by the Gompertz non-linear mixed model; food consumption was analyzed by the mixed effects ANOVA Model; and the prevalence of foot lesions and tumor rate were analyzed by the Chi-squared test. This analysis demonstrated that housing conditions (individual vs. paired and wire-bottom vs. solid-bottom) do affect body weight, food consumption, survival, foot lesion and tumor rates in two-year Sprague-Dawley rat carcinogenicity studies. The results provided a contrasting backdrop to a widely accepted dogma that solid-bottom housing is generally better than wire-bottom housing without consideration of individual vs. pair housing. Additionally, regarding solid-bottom housing, there are clear advantages to pair-housing compared to individual housing which may lead to lower food consumption and average body weight, decreased incidence of foot lesions, decreased total tumors rates in females, and increased survival in females.

**Poster Number:** T-57

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Skin

**KEEPING BALANCE OF ELOVL1 MAY BE IMPORTANT TO IMPROVE SKIN BARRIER**

I-H Bae, D-M Go, S-H Lee, and D-Y Kim
College of Veterinary Medicine, Seoul National University, Seoul, Korea

The lipid component in the stratum corneum is important for the epidermal permeability barrier and elongation of very chain fatty acid 1(ELOVL1) is one of critical regulators in the control of skin lipid contents. It is well known that knockout of ELOVL1 in mice resulted in impaired ceramide chain length in the skin and up-regulation of ELOVL1 improves inflamed skin condition. However, the molecular mechanism is not enough understood, especially in atopic dermatitis (AD). In the present study, we evaluated the change of ELOVL1 in the atopic dermatitis. To mimic atopic dermatitis, we used a hapten-induced, Th2-mediated AD murine model. Topical treatment of oxazolone as hapten induced atopic dermatitis-like phenotype in the skin and decreased the level of trans-epidermal water loss suggesting that skin barrier function impaired. Serum IgE and IL-4 mRNA increased and many amounts of T lymphocytes and eosinophils were infiltrated into the skin lesion, demonstrating this model can mimic Th2-mediated atopic dermatitis in human. Interestingly, we performed qPCR to evaluate the change of mRNA expression of ELOVL1. It was up-regulated 2.7 fold compared to normal control. Then, we evaluated the expression level of Liver X Receptor (LXR) which plays a role as a ELOVL1 agonist. LXR-beta
increased 3.3 fold in the skin lesion compared to normal control. These results are different from that down-regulation of ELOVL1 gene induced impaired function of skin barrier many studies have suggested. Keep balance of ELOVL1 expression may be important to improve skin barrier function in the atopic dermatitis.

§ Poster Number: T-58

Section: STP and Industrial and Toxicologic Pathology
Keyword: Industrial and Toxicologic Pathology

MENINGEAL THROMBOSIS ASSOCIATED WITH USE OF ELIZABETHAN COLLARS IN RATS

C. Hollinger1,2, J. Ibanes1, Z. Lloyd1, and K. Storves1
1MPI Research, Mattawan, MI, USA, 2Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA

The practice of toxicologic pathology requires accurate recognition of test article-related effects, which is complicated when there are confounding procedure-related findings. Following observation of non-dose related meningeal thrombosis in intravaginally-dosed rats, it was hypothesized that meningeal thrombosis was associated with study-specific procedural variables. To test this, a 28-day study was designed to evaluate two procedures: 6-hour post-dose E-collar placement and 1-minute post-dose inverted animal restraint. The four study groups each consisted of 20 adult female Sprague Dawley rats receiving once daily intravaginal doses of 0.1 ml of sterile surgical lubricant. The three test groups respectively received intravaginal dosing plus 1) post-dose animal inversion and E-collar placement, 2) post-dose animal inversion only, or 3) post-dose E-collar placement only. The control group received only intravaginal dosing with neither post-dose animal inversion nor E-collar placement. There were no differences in clinical observations, clinical pathology, organ-weights, or macroscopic findings between any groups. Microscopic thrombosis of varying chronicity was identified within the meninges, predominantly associated with the pineal gland, in 8 females (40%) exposed to E-collar placement only, in 6 females (30%) exposed to both inversion and E-collar placement, and in none of the females in inversion-only or control groups (Fisher’s exact for E-collar ± inversion, p < 0.05). In the collar-plus-inversion group, vascular findings also rarely involved the cerebral cortex. These findings support an association of meningeal thrombosis with E-collar use in rats, and have implications for future studies that apply restraints around the neck to prevent animal access to administered test substances.

Poster Number: T-59

Section: STP and Industrial and Toxicologic Pathology
Keyword: Immune System

AN UNIQUE POPULATION OF NATURAL KILLER CELLS IS ASSOCIATED WITH TRANSMURAL INFLAMMATION AND VASCULITIS IN THE ADOPTIVE T CELL TRANSFER MODEL OF COLITIS
I. Mikaelian, H. Knight, A. Schmidt, G.A. Kingsbury, J. Godwin, B. McRae, and J. Paez-Cortez
Abbvie Bioresearch Center, Worcester, MA

The adoptive T cell transfer model of colitis, in which CD4\(^+\)/CD45RB\(^hi\) T cells from the spleen of Balb/c mice are administered to severe combined immune deficient mice, is considered the most translational model of Crohn’s disease (CD). Colonic inflammation is characterized by gradual expansion of the lamina propria by T cells and inflammatory macrophages starting 10-14 days after T cell transfer. In addition, colonic erosions, ulcerations, transmural inflammation, vasculitis and lymphangitis may also occur 4-8 weeks post-transfer. In these severely inflamed areas, we identified large amoeboid cells with acidophilic granules, positive for granzyme B, CD45/RB220 and periodic acid Schiff, and negative for IBA1, F4/80, lysozyme, CD3, and MHCII. This panel identified these cells as natural killer (NK) cells and ruled out NKT cells. Concomitantly, a relatively abundant population of small intra-epithelial NK cells was also identified by immunohistochemistry. The numbers of both NK cell populations and the overall severity of inflammation decreased in mice upon administration of an anti-p40 IL-12/IL-23 antibody which is a reference intervention in this model. The translational relevance of this observation is uncertain because amoeboid NK cells were not identified in CD patient samples, including in the areas of ulceration, vasculitis, and lymphangitis.

Poster Number: T-60

Section: STP and Industrial and Toxicologic Pathology
Keyword: Urinary System

SPONTANEOUS UROLITHIASIS WITH DIVERTICULA OF UROTHELIUM IN TWO MALE SPRAGUE DAWLEY RATS

J.L. Grieves\(^1\), K.S. Regan\(^2\), S.M. Cohen\(^3\), R.M. Peters\(^1\), and M.E. Carsillo\(^1\)
\(^1\)Takeda Pharmaceuticals International Company, Cambridge, MA, \(^2\)Regan Path/Tox Services, Ashland, OH, \(^3\)University of Nebraska Medical Center, Omaha, NE

Urolithiasis is an uncommon finding in rat toxicology studies and is typically reported as an incidental finding at scheduled necropsy. Uroliths are commonly associated with hyperplasia of urothelium and rarely with diverticula formation. Distinguishing diverticula, downgrowths of urothelium into the urinary bladder wall, from carcinoma can be difficult. Here we report fatal spontaneous urolithiasis in two 5-6 month old Sprague Dawley rats that was associated with marked papillary hyperplasia of urothelium and diverticula. Both animals were found dead with a brief history of weight loss and red and/or yellow urogenital discharge prior to death. Macroscopic examination revealed the presence of calculi in the urinary bladder, ureters, and kidney, distended bladder, ureters, and kidney pelves, irregular kidney surface, and thickened urinary bladder. Microscopic examination revealed pyelonephritis and marked diffuse papillary hyperplasia of urothelium that was associated with the presence of cystic or cleft-like spaces in the urinary bladder wall that sometimes communicated with the mucosa and were lined by urothelium. The urothelial cells lining these spaces were hyperplastic, but did not exhibit dysplastic characteristics such as atypia or increased mitoses. Therefore, these structures were diagnosed as diverticula and were not considered neoplastic. This report expands our knowledge of the background incidence of uroliths in rats used in toxicology studies and highlights a rare hyperplastic lesion that can be mistaken for neoplasia.
QUANTITATIVE ASSESSMENT OF ACUTE KIDNEY INJURY

T. Friedman, H. Shankaran, D. Snow, D. Hughes, M. Blais, and K. Maratea

Drug-related kidney toxicity is a common, often preventable cause of acute kidney injury (AKI), which may lead to chronic kidney disease. Nephrotoxicity causes considerable patient morbidity, and is also a factor in failure of drug candidates preclinically. Advancement of more sensitive methods for prediction of kidney toxicity in preclinical studies is therefore important for both drug development and patient safety. A mathematical model integrating drug-induced histopathologic changes in the kidney and the temporal expression of renal biomarkers can be used to infer information on the mechanism of injury, and ultimately translate renal toxicity from preclinical species to humans. Toward this end, immunohistochemical expression of kidney injury molecule-1 (KIM-1) and the proliferation marker Ki-67 were quantified to assess proximal renal tubular damage and recovery, respectively, following exposure to repeated doses of nephrotoxic compounds in rats. Pharmacokinetic-pharmacodynamic (PK/PD) modeling was then used to quantitatively link plasma exposures to renal histolopathology scores, and to renal and urinary KIM-1 levels. The PK/PD model can be used for selecting doses and regimens that minimize nephrotoxicity. Further, the current work sets the stage for the development of systems pharmacology models for AKI that involve a more detailed mechanistic description of renal injury and recovery.

ATAXIN-2 (ATX2) IS AMONG 100 NEW REGULATORS OF IP3-DEPENDENT CA2+ SIGNALING IDENTIFIED THROUGH A GENOME-WIDE RNAI SCREEN IN DROSOPHILA S3 CELLS

E.J. Sung, G.S. Bird and S.B. Shears
Signal Transduction Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, USA

IP3-dependent Ca2+ signaling is a highly-conserved process that drives many biological processes, so its disruption underlies many pathological conditions, including several neurological disorders. To identify novel upstream regulators of IP3-mediated Ca2+ mobilization, we have used genetically-tractable Drosophila S3 cells in a high throughput RNAi screen. This approach was made possible by our recent discovery that a Drosophila cytokine, dGBP, activates receptor-dependent, IP3-mediated Ca2+ mobilization. We created a S3 cell line hosting a fluorescent Ca2+ sensor, GCaMP3. We used a genome-wide RNAi library from the Harvard/HHMI to screen almost 14,000 genes for their positive or negative impact upon dGBP-initiated Ca2+ mobilization. We identified 48 positive and 53 negative novel...
regulators. Interestingly, 17% of our negative regulators are already known to function in neurogenesis. The most significant among these is ataxin-2 (Atx2). This is striking because CAG expansion in the homologous human gene (ATNX2) has been associated with activation of Ca2+ signaling and neurodegeneration in Spinocerebellar Ataxia Type 2 (SCA2), a neurodegenerative disease. Nevertheless, current dogma posits that wild-type ATX2 has no role in Ca2+ mobilization by IP3 per se, and it is “gain-of-function” Ca2+ release that results from ATX2 acquiring an extended poly-Q tract. In contrast, our finding indicates wild-type Atx2 is a physiologically-relevant suppressor of Ca2+ mobilization. Thus, we open up new avenues for future research into SCA2 pathophysiology, by raising the possibility that some individuals might develop this condition as a result of genetic disruptions that perturb wild-type ATX2 expression and activity, even in the absence of CAG expansion.

**Poster Number:** T-63

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Neoplasia

**SPONTANEOUS TUMORS IN COMMON LABORATORY ANIMAL SPECIES USED IN 1-, 3- AND 6-MONTH NONCLINICAL SAFETY STUDIES**

S. Beazley and C. Dean
MPI Research, Mattawan, MI

Spontaneous neoplasia in laboratory animals is not restricted to older animals on 2-year carcinogenicity studies. References on the tumor types and incidence rates occurring during shorter term studies can help differentiate spontaneous/incidental tumors from those induced by the test article; however, the availability of this data is limited. A retrospective evaluation of historical control data compiled from 2005-2015 at a Contract Research Organization was performed to determine type and incidence of neoplastic lesions occurring in animals on 1 month, 3 month and 6 month duration studies in Sprague-Dawley rats, CD-1 mice, Beagle dogs, and cynomolgus monkeys. Some of the most frequent tumors by species consisted of: lymphoma, amphophilic-vacuolar renal tubule adenomas and carcinomas, nonglandular stomach squamous cell carcinomas and papillomas, mammary gland adenocarcinomas, pars distalis adenomas, and thyroid follicular and C-cell adenomas in rats; lymphoma and bronchiolar adenomas in mice; and skin histiocytomas in dogs. There were no neoplasms identified in cynomolgus monkeys.

§ **Poster Number:** T-65

**Section:** STP and Industrial and Toxicologic Pathology

**Keyword:** Neoplasia

**CHARACTERIZATION OF THE TUMOR SPECTRUM ARISING IN HZE ION IRRADIATED OUTBRED MICE**

E.F. Edmondson ¹, ², D.A. Kamstock ³, ⁴, C.M. Fallgren ², and M.M. Weil ²
Space travel exposes astronauts to unique forms of densely ionizing radiation known as HZE ions. Ionizing radiation is efficient at causing DNA damage and neoplasia, however, the differential ability of HZE ions to produce neoplasia and metastatic disease is largely uncharacterized. Inbred, genetically identical mice are traditionally used for this type of study, however genetically heterogenous mice are more similar to human populations and allow for genetic mapping of susceptibility loci for complex traits, such as neoplasia and metastasis. In the present study, Heterogenous Stock (HS) outbred mice were exposed to 28Si ions (n=308), 56Fe ions (n=314), 137Cs γ rays (n=615), or sham irradiation (n=613) and monitored twice daily for 800 days. Thorough necropsy examinations were performed on each mouse and all organ systems and each detected tumor was characterized histologically. For lymphoid neoplasms and nonlymphoid round cell neoplasms, peripheral complete blood counts and marrow tissue were analyzed to reveal leukemic phases. For solid tumors, such as Harderian gland adenocarcinomas and hepatocellular carcinomas, lung fields were examined grossly and histologically to detect pulmonary micrometastases. Irradiated mice, regardless of the quality of radiation, had decreased survival compared to unirradiated controls. Mice exposed to 3 Gy γ-rays had decreased survival compared to mice exposed to 0.4 Gy iron or silicon nuclei. The spectrum of tumors observed in HZE ion irradiated animals was unique in comparison to γ-irradiated mice, which supports the hypothesis that there are mechanistic differences in carcinogenesis or differential efficiencies of tumorigenesis following irradiation with HZE ions in mice.

**Poster Number:** T-66

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Industrial and Toxicologic Pathology

**BODY WEIGHT DECREASE IS ASSOCIATED WITH A GREATER VARIATION IN THE WEIGHT OF PITUITARY GLAND AND REPRODUCTIVE ORGANS**

C. Amuzie, C. Dykstra, and S. Denham

STP best practice guidelines recommend that organ weights (OW) for liver, heart, kidneys, adrenal glands, brain, thyroid glands, pituitary glands, and testes be collected for rats in all multi-dose GLP studies that last up to 365 days. These OW are used to determine potential test article-related effects, partly by statistically analyzing whether OW variations are due to chance. However, the impact of body weight (BW) decrease on OW variations has not been evaluated with large data. We hypothesized that BW decrease affects different OW variably under the same study conditions. Over six hundred studies of ≤6-month were reviewed; and 170 were selected based on ≥5% decrease in BW of treatment groups relative to controls. Scatterplots that related the percent OW changes to their respective percent BW changes were created. Gender, age, and housing were also included as confounding variables in the predictive model. Multiple linear regression analyses were run on the model; with different levels of BW decrease relative to the control. The results indicate that 30 to 50% of the OW variations in the pituitary gland and reproductive organs are related to BW decrease. The result for pituitary gland is intriguing because weight changes in this master endocrine organ could be a concern to regulators and
stakeholders. Overall, the data suggest that caution should be exercised when interpreting reproductive OW changes in a BW decrease scenario.

**Poster Number:** T-67

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Other

**A COMPARISON OF NEGATIVE AND POSITIVE CONTROL TUMOR INCIDENCE IN RASH2 MICE WITH PREVIOUSLY PUBLISHED DATA**

M. Morse, B. Jacob, A. Sargeant, and K. Bonnette  
Charles River Laboratories, Spencerville, OH

Under ICS S1B, a 6-month carcinogenicity study in rasH2 (CBByB6F1-Tg(HRAS)2JIC) mice is accepted by regulatory agencies as an alternative to a 2-year carcinogenicity study in mice. To determine if our facility’s background incidence of neoplastic findings in 6-month rasH2 mouse studies is within expected limits, we compared the incidence of spontaneous and induced tumors in multiple studies conducted in our facility with those previously published by Nambiar et al. (Pfizer) and Paranjpe et al. (BioReliance). For untreated or vehicle (negative) control mice at our facility, the most common spontaneous neoplasms in males and females were lung adenomas (7.6%, males; 5.3%, females), followed by splenic hemangiosarcomas (2.5%, males; 3.9%, females), lung adenocarcinomas (2.2%, males; 2.8%, females), squamous cell papillomas of the stomach (1.4%, males; 0.7%, females), and Harderian gland adenomas (1.1%, males and females). For rasH2 mice administered N-nitrosomethylurea as a positive control, the most common neoplasms were lymphomas (68.0%, males; 74.7%, females) and squamous cell papillomas of the stomach (72.0%, males; 64.0%, females), followed by squamous cell papillomas of the skin (17.3%, males; 43.2%, females), adenocarcinomas of the small intestine (13.3%, males; 8.0%, females), lung adenomas (9.3%, males; 16.0%, females), squamous cell carcinomas of the stomach (10.7%, males; 13.3%, females), and adenomas of the small intestine (5.3%, males; 9.3%, females). These spontaneous tumor incidences in negative control rasH2 mice and induced tumor incidences in positive control rasH2 mice are within the tumor incidence ranges of the Pfizer and BioReliance databases.

**Poster Number:** T-68

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Other

**VETERINARY PATHOLOGIST INVOLVEMENT IN EXPERIMENTS USING HUMAN TISSUE IN PHARMACEUTICAL RESEARCH AND DEVELOPMENT**

C. Fishman and R. Adler  
GlaxoSmithKline, King of Prussia, PA

Failure of drug development candidates to show efficacy in Phase II clinical trials is perceived as a major cause of inefficiency and excess cost in drug development. Improvement of the success rate at this stage
of drug development, through better understanding the human disease process and the molecular drug targets, is a strategic focus of pharmaceutical companies. Emerging in vitro technologies are providing opportunities to increase the use of human biological samples (HBS) in experiments, as adjuncts to animal models; thereby further enabling species and disease-relevant assays. The types of HBS used in pharmaceutical research include immortalized cell lines, isolated primary cells, induced pluripotent stem cells and their differentiated lineages, biofluids and fixed, frozen or fresh human tissue. An expanding array of assay types, including biochemical, morphological and “omics” from regulome to metabolome, and emerging technologies such as organoids and other engineered tissue culture systems, are being applied to HBS. Where human tissues are used, a pathologist provides important input into assessment of sample quality and suitability, assay validation, study design and results generation, interpretation and integration; similar to the traditional role of the veterinary pathologist in animal studies. Because veterinary pathologists have long-standing roles in pharmaceutical companies in both safety and efficacy, they are well-positioned to provide support to investigators using human tissues. Creating a framework for the use of human tissue in research, based on an understanding of informed consent, and distinctions between diagnostic and research pathology, allows the veterinary pathologist to provide needed expertise to investigators.

**Poster Number:** T-69

**Section:** STP and Industrial and Toxicologic Pathology  
**Keyword:** Other

**CHALLENGES AND SOLUTIONS FOR MAPPING PATHOLOGY DATA TO SEND**

M. Wasko, F. Mura, R. Buchanan, and L. Kaufman  
PDS Life Sciences, Mt Arlington, NJ

Based on our experience creating SEND datasets for FDA submission, we can group SEND mapping challenges for pathology into several patterns: 1) inconsistent or incomplete data entry for gross and microscopic pathology findings; 2) inconsistent use of units for clinical pathology findings; 3) inconsistent use of terminology for clinical pathology (use of the same term to mean different things in different parts of a dataset); and 4) uncertainty of which domain to map biomarkers. For all of these disciplines, it is critical that data capture be conducted in a consistent manner so that mapping can be automated, since manual solutions are impractical in a production environment. For example, microscopic findings as collected by the pathologist, including base pathological processes and modifiers, are placed into one SEND variable (MIORRES) in the Microscopic Domain (MI). The base pathological process and different modifiers get mapped again to different SEND variables, some of which require controlled terminology. Without a consistent way to record data across studies at the time of data capture, it is difficult to automate this type of mapping. It is also critical that terminology be used in a consistent fashion for accurate mapping to controlled terminology. In conclusion, the use of SEND presents new challenges for data capture while at the same time creates opportunities for analyzing large quantities of data in ways that are not currently feasible.

**Poster Number:** T-70
INHAND AND COLLABORATION WITH THE FDA ON SEND - BACKGROUND AND CURRENT STATUS

C.M. Keenan¹, J. Baker², A. Bradley³, D.G. Goodman⁴, T. Harada⁵, R. Herbert⁶, W. Kaufmann⁷, R. Kellner⁸, B. Mahler⁹, E. Meseck¹⁰, T. Nolte¹⁰, S. Rittinghausen⁸, J. Vahle¹¹, and K. Yoshizawa¹²
¹CM Keenan ToxPath Consulting, Doylestown, PA, ²Charles River, Frederick, MD, ³Charles River, Tranent, Scotland, UK, ⁴Independent Consultant, Potomac, MD, ⁵The Institute of Environmental Toxicology, Jososhikai, Ibaraki, Japan, ⁶NIEHS, Research Triangle Park, NC, ⁷Merck KGaA, Darmstadt, Germany, ⁸Fraunhofer ITEM, Hannover, Germany, ⁹Novartis Institute for Biomedical Research, East Hanover, NJ, ¹⁰Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany, ¹¹Eli Lilly & Company, Indianapolis, IN, ¹²Kansai Medical University, Hirakata, Osaka, Japan

The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) manages the overall objectives of the project and the development of harmonized terminology for each organ system is the responsibility of the Organ Working Groups (OWG), drawing upon experts from North America, Europe and Japan.

Great progress has been made with 9 systems published to date – Respiratory, Hepatobiliary, Urinary, Central/Peripheral Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands in Toxicologic Pathology and the Integument and Soft Tissue and Female Reproductive System in the Journal of Toxicologic Pathology as supplements and on a web site – www.goReni.org. INHAND nomenclature guides offer diagnostic criteria and guidelines for recording lesions observed in rodent toxicity and carcinogenicity studies. The guides provide representative photo-micrographs of morphologic changes, information regarding pathogenesis, and key references.

During 2012, INHAND GESC representatives attended meetings with representatives of the FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and the National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to begin incorporation of INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. The interest in utilizing the INHAND nomenclature, based on input from industry and government toxicologists as well as information technology specialists, suggests that there will be wide acceptance of this nomenclature.

Poster Number: T-71

"ADVERSITY" IN TOXICITY STUDIES, THE PATHOLOGISTS' POINT OF VIEW – RESULTS FROM THE 4TH ESTP INTERNATIONAL EXPERT WORKSHOP IN PARIS, JUNE 8-9, 2015
B. Lenz, W. Kaufmann, X. Palazzi, G. Pohlmeyer-Esch, and ESTP Adversity Working Group

There is clearly a demand for better alignment in the toxicologic pathology community regarding nonclinical "adversity" as it impacts the development process of chemical entities and new drugs. Publications about general principles of "adversity" and NOAEL assignments in toxicology are numerous, but do not offer sufficient practical guidance to the study pathologist.

The European Society of Toxicologic Pathology (ESTP) therefore recently tasked a workshop to a) review available definitions of "adversity"; b) weigh determining and qualifying factors of "adversity" based on anonymized case examples; and c) recommend a practical approach, yet still using rigorously all biological information, to define "adversity" in toxicology reports.

22 international expert pathologists and toxicologists from the pharmaceutical and chemical industry, contract research organizations and regulatory authorities met during five preparatory teleconferences and a 1.5 day workshop in Paris.

Experts insisted that a holistic, weight-of-evidence, case-specific approach should be followed for each "adversity" assessment. A working definition of "adversity" to be used by pathologists was developed. Based on information/tools available to pathologists, "adversity" will be typically determined at a morphological level (most often the organ) in the pathology report and will refer to the test species. Final "adversity" calls and integration of target pharmacology information and consideration of human translation should be made in the toxicology overview in the dossier. Differences in interpretation and consequences of "adversity" between (agro)chemical and pharmaceutical industries and among world regions were emphasized. The results of this workshop are complementary to the recommendations drafted by the STP SRPC committee and a valuable prerequisite for organ- or lesion-specific workshops planned to be organized by the ESTP.