2024 ACVP/ASVCP Annual Meeting Accepted Abstracts – Platform and Poster

Clinical Pathology Abstracts

Chair: Amy MacNeill

Sunday, November 17, 2024
02:30 PM – 02:45 PM

SERUM PROTEIN ELECTROPHORESIS FINDINGS IN HEALTHY CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS)

Valerie Wong, Corie Drake, Michelle Rivard, Katherine Prager, Jessica Hokamp, Rachel Cianciolo, Pádraig Duignan, Margaret Martinez

Background: While reference values for routine hematology and clinical chemistry analytes have been reported for wild and stranded California sea lions (CSLs), reference values for protein fractions as determined on serum protein electrophoresis (SPE) are not available.

Objective: The objective of this study was to determine the reference intervals of protein fractions as determined on SPE for clinically healthy CSLs.

Methods: During August 2015 – May 2023, blood samples were collected from 136 clinically healthy CSLs. Serum was harvested and frozen until analysis. Total protein concentrations were determined using the Beckman Coulter AU680 chemistry system and reagents. Protein electrophoresis was performed on the serum samples using the Sebia Hydrasys 2 system. Protein fractions were assigned by visualization of the densitometry tracings. The reference intervals (RIs) were generated using Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: The RI for total protein concentration was 6.4 – 9.0 g/dL. The RI for albumin concentration was 2.8 – 4.4 g/dL. All samples (n = 136) showed an alpha-1 globulin (RI: 0.2 – 0.4 g/dL), alpha-2 globulin (RI: 0.2 – 0.5 g/dL), alpha-3 globulin (RI: 0.5 – 1.0 g/dL), beta-1 globulin (RI: 0.4 – 1.4 g/dL), and gamma globulin (RI: 0.8 – 2.6 g/dL) fractions. Fifty-four samples (n = 54) showed a beta-2 globulin fraction (RI: 0.2 – 0.9 g/dL).

Conclusions: All animals in this study showed an alpha-3 globulin fraction, but the beta-2 globulin fraction was not a consistent finding.

Sunday, November 17, 2024
02:45 PM – 03:00 PM

THE CYTOLOGICAL FEATURES OF FELINE MERKEL CELL CARCINOMA IN 6 CASES

Nao Akiyama-Nagao[1], Minami Goto[1], Hiroko Hiraoka[1], Ayumi Sumi[1], Nozomi Shimonohara[1], Rina Nabeta[1,2]
[1]IDEXX Laboratories, Tokyo, Japan, [2]Tokyo University of Agriculture and Technology, Tokyo, Japan

Background: Merkel cell carcinoma is a very rare skin neoplasm. There are limited reports that describe cytological findings of the disease. The aim of this study is evaluating the cytological features of feline Merkel cell carcinoma.

Materials and **Methods:** The glass slides of fine-needle aspirated sample from six feline Merkel cell carcinomas were examined. The diagnoses of all cases involved in this study were confirmed by histopathology and immunohistochemistry.

Results: Two cytologic subtypes were identified: round cell (3/6) and epithelial/neuroendocrine (3/6). In the round cell subtype, the neoplastic cells were discrete round cells, which often appeared individually. Cells exhibited typical lymphocytic or histiocytic morphology with high or variable N/C ratio, round nuclei, coarse chromatin, and prominent nucleoli. Binucleation or multinucleation were occasionally observed. In the epithelial/neuroendocrine subtype, cohesive clusters or cords of basaloid cells were predominant, which had high N/C ratio, scant basophilic cytoplasm, round to oval nuclei, coarse chromatin, and prominent nucleoli. Frequent nuclear molding was seen.

Discussions: Previous reports of feline Merkel cell carcinomas only described lymphocytic features on cytology. Interestingly, one of our cases showed rather histiocytic features than lymphocytic. Moreover, three of the six cases in this study had epithelial/neuroendocrine cytological appearance which has not reported previously to the best of our knowledge.

Conclusions: Feline Merkel cell carcinomas could have a variety of cytological features from round cell to epithelial/endocrine subtypes. Merkel cell carcinoma should be considered as a differential diagnosis for a feline cutaneous lesion with these characteristic cytological features

Sunday, November 17, 2024
03:30 PM – 03:45 PM

CYTOLOGICAL CHARACTERIZATION OF BILLUPS' BODIES IN A DOG WITH PULMONARY HYALINOSIS

Marta Gruarin[1], Catherine Ennet[1], Alba Bello[2], Amelia Tingey[1], Molly Reed[1], Carlo Bianco[1]
[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, United Kingdom, [2]IVC Evidensia Iberia, Madrid, Spain

Background: A 10-year-old female neutered Cockapoo was presented for post-mortem examination after humane euthanasia due to severe neurological signs associated with a portosystemic shunt. On gross examination, multifocal firm masses were noted in the lungs lobes and suspected to be neoplastic in origin.

Objective: The aim of the investigation was to determine and characterize the nature of the lung lesions.

Methods: Post-mortem smears alongside histopathology and histochemistry of the lung lesions were carried out. The cytology and histology preparations were examined using bright field, polarised light and epifocal fluorescent microscopy.

Results: The cytological preparations showed moderate numbers of irregularly round (20-50 µm in diameter), refractile, pale amphophilic structures, resembling glove powder, often containing a central irregular mark or fissure and accompanied by mixed neutrophilic and macrophagic inflammation with occasional multinucleated giant cells.

The histopathological, histochemical, and optical features were consistent with pulmonary hyalinosis, and the structures observed on cytology were compatible with Billups’ bodies.

On cytological examination these structures additionally displayed peripheral birefringence under polarized light and autofluorescence. The fluorescent microscopy of the histopathology sections was unrewarding.

Conclusions: Pulmonary hyalinosis is an uncommon type of alveolar filling disorder which may represent a diagnostic pitfall mimicking neoplasia macroscopically. This report represents the first cytological description of the appearance of pulmonary hyalinosis with Billups' bodies in a dog, highlighting the usefulness of cytology in the identification of this condition.

Acknowledgments: Scott Hulme and Emma Pritchard, School of Veterinary Medicine and Science, Nottingham, UK.

Sunday, November 17, 2024
03:45 PM – 04:00 PM

EVALUATION OF SERUM YKL-40 IN DOGS WITH MULTICENTRIC LYMPHOMA

Chien-Chun Kuo, Shang-Lin Wang, Yuan-Yuan Xia, Jih-Jong Lee, Wei-Hsiang Huang, Tai-Ching Liao
National Taiwan University, Taipei, Taiwan

Background: YKL-40, a glycoprotein, is a known prognostic biomarker in human cancers. However, its prognostic role in canine multicentric lymphoma prognosis has not been evaluated.

Objective: To investigate serum YKL-40 levels in dogs with multicentric lymphoma and its associations with disease characteristics and outcomes.

Methods: Serum YKL-40 levels in dogs with intermediate to large cell multicentric lymphoma and healthy controls were measured using ELISA. Pre-treatment serum YKL-40 levels were compared with clinical parameters and outcomes. Serial serum samples before, during, and after chemotherapy underwent analyzed for longitudinal analysis.

Results: Pre-treatment serum YKL-40 levels were significantly higher in the dogs with multicentric lymphoma (n=30) compared to healthy dogs (n=11) (p<0.01). A serum YKL-40 of 445.1 pg/mL had a 100% positive predictive value for confirmation of lymphoma. Dogs at clinical stage V had the highest median serum YKL-40 levels (p=0.03). YKL-40 levels before treatment did not correlate with age, sex, immunophenotype, substages, progression-free survival (PFS), or overall survival (OS). Longitudinally, YKL-40 levels decreased after completion of chemotherapy (p=0.03). Univariate COX analysis showed that body weight, clinical stage, substage, and leukocytosis were associated with PFS, while leukocytosis was associated with OS. YKL-40 levels were not associated with PFS (p=0.83) or OS (p=0.27).

Conclusions: Serum YKL-40 levels are significantly elevated in dogs with multicentric lymphoma, especially at advanced clinical stages, but do not correlate with immunophenotype, disease progression, PFS, or OS. Serum YKL-40 levels should be investigated as a potential indicator of response to lymphoma treatment or disease recurrence.

Sunday, November 17, 2024
04:00 PM – 04:15 PM

USE OF PLASMA CELL-FREE DNA FOR DIAGNOSIS OF LYMPHOMA IN DOGS: A PROOF-OF-CONCEPT STUDY

Sai Navya Vadlamudi, Priscila B.S. Serpa
Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA

Background: Lymphoma is a common hematopoietic malignancy in dogs. Diagnosing and characterizing the disease can be invasive, expensive, and complicated by tumor heterogeneity. Studies claim that genetic aberrations in solid tumors in humans can also be found circulating in

plasma cell-free DNA (cfDNA). However, no studies have demonstrated that cfDNA can be used for genotyping canine lymphoma.

Objective: Evaluate the feasibility of using cfDNA and Next-generation sequencing (NGS) to identify mutations in dogs with B cell lymphoma.

Method: Five dogs diagnosed with B cell lymphoma through flow cytometry and/or PAAR were enrolled. Samples collected included EDTA-anticoagulated blood for plasma (cfDNA), tumor fine-needle aspirate smears (tumor DNA), and buccal swabs (genomic DNA-germline control). After DNA purification and whole genome sequencing, NGS analysis was performed to detect somatic variants using various bioinformatics tools.

Results: Our results revealed many shared somatic variants between matched cfDNA and tumor DNA, with 1.7-50% of tumor variants also found in corresponding plasma samples. Shared variants constituted only 0.5-10% of all plasma somatic variants. We were unable to identify all lymphoma-specific mutations present in the tumor samples within the corresponding plasma samples. Additionally, there were many unique mutations found exclusively in either the plasma or the tumor samples.

Conclusion: Although tumor-specific mutations can be detected in plasma, cfDNA may not capture the full genetic heterogeneity of tumors when plasma volume is limited. The low proportion of tumor-derived DNA in plasma limits the detection of all relevant mutations, highlighting the need for further optimization of cfDNA-based techniques.

Sunday, November 17, 2024
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DEEP LEARNING-ASSISTED CANINE LYMPHOMA CLASSIFICATION

Chanel Shum, Donghee Lee, Hyunji Jo, Joanne Kim, Heon Heo, Shir Gilor, Christopher Lanier, Kevin Hall, Michael Dark, Jong Hyuk Kim, Cleverson de Souza
University of Florida, Gainesville, FL, USA

Background: Canine lymphoma (cLSA) exhibits significant heterogeneity, complicating diagnosis and treatment. Conventional diagnostic methods, such as cytology and histopathology, can be time-consuming and require expertise that may not always be available.

Objectives: This study aimed to harness machine learning, specifically Convolutional Neural Networks (CNN), to classify cLSA and its subtypes.

Methods: A total of 2,618 images were used: 1,090 from B-cell LSA across 68 patients, 490 from T-cell LSA across 35 samples, and 1,038 from reactive lymphoid hyperplasia (RLH) across 77 patients. A transfer learning approach with a pre-trained ResNet-50 CNN was employed, modified for sequential binary classification tasks: distinguishing RLH from LSA, and classifying T-cell and B-cell malignancies. Ten-fold cross-validation assessed accuracies and losses from training.

Results: For LSA vs. RLH classification, the model achieved 98.6% training accuracy, 0.983 precision, 0.983 recall, an F1 score of 0.983, and 1.00 AUC over 10 epochs. Cross-validation showed 92.5% average accuracy, 0.184 average training loss, 92.2% average validation accuracy, and 1.226 average validation losses. For B-cell vs. T-cell classification, it achieved 94.8% training accuracy, 0.935 precision, 0.934 recall, an F1 score of 0.935, and 0.98 AUC. Cross-validation showed 75.1% average accuracy, 0.508 average training loss, 75.9% average validation accuracy, and 0.810 average validation losses.

Conclusions: This study demonstrates the potential of deep learning to classify LSA and its subtypes using cytology images. Future work includes investigating model decision-making, validating with other CNN architectures, and expanding datasets

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EXPLAINABLE ARTIFICIAL INTELLIGENCE FOR CYTOLOGICAL ANALYSIS OF CANINE LYMPHOMA

Donghee Lee, Chanel Shum, Hyunji Jo, Joanne Kim, Heo Heon, Shir Gilor, Christopher Lanier, Kevin Hall, Michael Dark, Cleverson Cleverson, Jong Hyuk Kim
University of Florida, Gainesville, FL, USA

Background: Lymphomas are among the most prevalent tumors in dogs. Cytological and histological evaluation remain the primary method for lymphoma diagnostics. Despite the standardization of diagnostic criteria, morphological assessment still relies on human perception, leading to potential discrepancies and inconsistencies across specialized individuals. The Convolutional Neural Network (CNN) is a robust artificial intelligence tool that facilitates biomedical applications.

Objective: The objective of this study was to evaluate the performance of multiple CNN models in determining lymphoid malignancy and immunophenotyping B-cell (BCL) and T-cell lymphoma (TCL) using cytological images. The study also aimed to assess the explainability of the decision-making process in the models.

Methods: A total of sixteen pretrained CNN models were deployed, including AlexNet, ConvNeXt, GoogleNet, ResNet-18, -34, -50, -101, -152, Inception V3, ShuffleNet V2, VGG-16, -19, SwinTransformer_b, SwinTransformer_v2_b, VisionTransformer_16, and VisionTransformer_32. Grad-CAM was employed to visualize the cytological features used in the decision-making processes of each model.

Results: Our results revealed that the highest training accuracy across the CNN models ranged from 0.88 to 1.00 for classifying lymphoma and reactive lymphoid hyperplasia (RLH), with test accuracy ranging from 0.86 to 0.99. For the classification of BCL and TCL, the highest training accuracy across the models ranged from 0.80 to 0.99, with test accuracy ranging from 0.74 to 0.96. Grad-CAM visualization heatmaps from cytological images highlighted morphological features, which varied across the CNN models.

Conclusions: This study offers a comprehensive evaluation of CNN models in cytology-based lymphoma diagnostics, highlighting the explainability of morphological features relevant to pathology.

Sunday, November 17, 2024
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VETCLINPATHGPT: EXPLORING THE POTENTIAL OF GENERATIVE ARTIFICIAL INTELLIGENCE IN VETERINARY EDUCATION

Candice Chu
Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Background: Customized Artificial Intelligence (AI) chatbots, like ChatGPT, have potential applications in veterinary education, but their use has yet to be extensively explored. This study describes the development of “VetClinPathGPT,” a custom GPT designed for teaching and assisting in veterinary clinical pathology.

Objective: To develop "VetClinPathGPT," a tailored AI tool that integrates information from selected textbooks and verified online sources to enhance learning in veterinary clinical pathology.

Methods: VetClinPathGPT was programmed using OpenAI's platform with configurations tailored for veterinary education, excluding image generation and code interpretation functionalities. The AI integrates content from three open-access textbooks with Creative Commons licenses: Clinical Veterinary Diagnostic Laboratory, Veterinary Histology, and The Lymphatic System of the Dog. To provide accurate, relevant information, web browsing was confined to the eClinPath website.

Results: Since its launch on May 14, 2024, VetClinPathGPT has not only facilitated over 1000 interactive sessions but also provided precise and comprehensible explanations on a range of practical topics. These include diagnostic strategies for anemia and azotemia, and the procedural steps for blood smear reviews with platelet estimates. It utilizes a dual approach, employing technical terms for professional users while offering simpler explanations to aid beginners' understanding.

Conclusions: VetClinPathGPT (https://chatgpt.com/g/g-rfB5cBZ6X-vetclinpathgpt) is a valuable educational tool for veterinary students and professionals. It demonstrates the utility of AI in enhancing the learning experience in veterinary clinical pathology. The success of this initial deployment encourages further research into the application of tailored GPTs in veterinary education.

Tuesday, November 19, 2024
09:00 AM – 09:15 AM

UTILIZING WHOLE EXOME SEQUENCING TO DESIGN A COST-EFFECTIVE TARGETED GENE PANEL TO ASSESS THE PREVALENCE AND CLINICAL SIGNIFICANCE OF MUTATIONS IN CANINE CD34+ ACUTE LEUKEMIAS

Jillian Nolan, Emily Rout, Kenzie Olsen, Anne Avery, R. Adam Harris
Colorado State University, Fort Collins, CO, USA

Background: Acute leukemia (AL) is a heterogeneous group of tumors with a poor prognosis in dogs, lacking extensive molecular characterization and clinical outcome data.

Methods: We utilized whole exome sequencing (WES) at 300x coverage to identify candidate mutations in 102 canine CD34+ AL samples diagnosed by flow cytometry. Somatic variant calling followed GATK best practices. A custom targeted sequencing panel was designed based on WES results. Medical records were reviewed from a separate cohort of dogs diagnosed with CD34+ AL by flow cytometry. Acute leukemia subtype was subclassified as myeloid, lymphoid, or unclassifiable using published criteria.

Results: WES identified at least one variant in the RTK/RAS pathway in over 75% of samples, with recurrent point mutations predicted in NRAS and KRAS. A total of 184 genes were prioritized from the WES data for the targeted sequencing panel. To date, medical records for 71 of 267 cases have been reviewed. CD34+ acute leukemias, regardless of subtype, showed poor prognosis in dogs, with a median survival time of 27 days (range: 0-335). No drug regimen proved particularly effective, with median survival times of 63 days for Prednisone +/- L-asparaginase and 48 days for maximum tolerated dose (MTD) chemotherapy. DNA was available in 198 cases for targeted sequencing and mutational analysis.

Conclusions: Acute leukemias in dogs are aggressive, heterogeneous neoplasms with mutations that overlap with those in human disease. These findings highlight the need for further molecular characterization and the development of more effective treatment strategies to improve outcomes for canine AL patients.

Tuesday, November 19, 2024
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EXTRAMEDULLARY ACUTE MYELOID LEUKEMIA INVOLVING PERIPHERAL LYMPH NODES OF DOGS: A RETROSPECTIVE STUDY OF 23 CASES

Jaspreet Kaur[1], Gabriella Diamantino[2], Katherine Morrison[2], Kristina Meichner[3], Nora L. Springer[4], Martha Hoffman[1], Dorothee Bienzle[2], Tracy Stokol[1]
[1]College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [2]Ontario Veterinary College, Guelph, ON, Canada, [3]College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [4]College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Background: Acute myeloid leukemia (AML) is considered a blood and bone marrow disease but can infiltrate extramedullary sites, which we have termed extramedullary AML (eAML). While AML infiltrates in lymph node aspirates have been described, there are few reports with details on morphology, immunophenotype and outcome.

Objective: To identify clinicopathologic features supporting a cytologic diagnosis of AML in peripheral lymph node aspirates from dogs.

Methods: Medical records of 23 dogs with a diagnosis of AML and archived lymph node aspirate smears from 2016-2024 were reviewed. Inclusion criteria were: >50% combined myeloid blasts and differentiating myeloid cells in smears and confirmation of myeloid lineage by phenotyping.

Results: Peripheral lymphadenopathy was the reason for presentation in 9 dogs (39%) or found incidentally on physical examination in 14 dogs (61%). Major hematologic findings included bi- or pancytopenia (18/23, 78%) and circulating blasts (18/23, 78%). Initial interpretations of lymph node aspirates were hematopoietic neoplasia (10/21, 48%), lymphoma (6/21, 29%), AML (3/21, 14%), lymphoid hyperplasia (1/21, 5%), and granulocytic precursor infiltrates (1/21, 5%). On repeat (n=21) or initial (n=2) evaluation of lymph node smears, cytologic features supporting eAML were >20% blasts with myeloid features, promonocytes and differentiating granulocytes, myeloid dysplasia, and a variable proportion of residual lymphocytes. Median survival was 22 days (range, 1-360 days), with significantly longer survival in dogs receiving chemotherapy (72 vs 22 days, p=0.039).

Conclusions: Our study highlights the importance of considering abnormal hematologic findings and identifying immature myeloid cells in lymph node smears to indicate possible eAML.

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AN ANTIBODY AGAINST CD80 BINDS TO TUMOR CELLS IN DOGS WITH ACUTE MYELOID LEUKEMIA BUT NOT B OR T CELL NEOPLASMS USING FLOW CYTOMETRY

Tracy Stokol, Sophie Thomas, Martha Hoffman, Shay Zhao
Cornell University, Ithaca, NY, USA

Background: CD80 is a T cell co-stimulatory molecule expressed on monocytes and dendritic cells. In preliminary trials with flow cytometric analysis, we found that a hamster anti-murine CD80 antibody (clone 16-10A1, RRID: AB_469417) labeled neutrophils and monocytes in canine blood submitted for routine hemograms.

Hypothesis: CD80 is a sensitive and specific flow cytometric marker for acute myeloid leukemia (AML) in dogs.

Methods: We first evaluated the antibody's cellular staining pattern in blood and bone marrow aspirates from healthy dogs, using flow cytometric analysis of unsorted and flow cytometric- or

immunomagnetic bead-sorted leukocyte subsets (neutrophils, monocytes, T cells, B cells) and examination of modified Wright's-stained smears. We then included the antibody in immunophenotyping panels applied to blood and tissue samples from 112 dogs with hematopoietic neoplasms.

Results: The anti-CD80 antibody bound to mature and immature neutrophils and monocytes, but not lymphocytes or eosinophils, in blood and bone marrow from healthy dogs. In dogs with hematopoietic neoplasms, the antibody did not label tumor cells in dogs with B (n=37) or T (n=36) lymphoma/leukemia but did bind to tumor cells in 72% of 39 dogs with AML. In dogs with AML, the proportion of tumor cells labeled with the anti-CD80 antibody was higher than with antibodies against other myeloid-associated antigens (CD4, CD11b, CD11c, CD14 and CD18 [clone YFC118]).

Conclusions: When using this antibody, CD80 is a sensitive and specific marker for AML in dogs. The antibody would be useful to include in immunophenotyping panels for classifying canine hematopoietic neoplasms.

Tuesday, November 19, 2024
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PERIPHERAL BLOOD LEUKOCYTE IMMUNOPHENOTYPIC CHANGES AND EVOKED CYTOKINE PRODUCTION ASSOCIATED WITH AGING IN THE DOG

R. Adam Harris, Emily Rout, Kenzie Olsen, Cora Contreras, Anne Avery
Colorado State University, Fort Collins, CO, USA

Background: Aging in humans is often correlated with significant changes in peripheral blood leukocyte phenotypes and overproduction of pro-inflammatory cytokines. Similar data for dogs is limited but could reveal comparable aging patterns.

Methods: Cross-sectional (n=905) peripheral blood samples were collected, and complete blood counts and immunophenotyping via flow cytometry were performed. The study included dogs of various breeds, ages, and health statuses. Dogs were categorized into life stages—puppy, young adult, mature adult, and senior—based on weight and age criteria from the Dog Aging Project. Leukocyte production of pro-inflammatory cytokines and chemokines was measured in 74 dogs via Miltenyi's Canine Cytokine Array and Luminex xMAP system after culturing whole blood with lipopolysaccharide (LPS) or without stimulation for 24 hours.

Results: Total white blood cell concentrations decreased in advanced life stages, primarily due to reductions in CD4+ T cell and CD21+ B cell counts (ANOVA $p<0.05$). Neutrophil and monocyte counts remained stable across life stages. Additionally, CD18 expression on monocytes and major histocompatibility complex class II (MHCII) expression on CD4+ T cells increased with age, indicating immune cell activation. Senior dogs' peripheral blood leukocytes produced more TNF-α upon LPS stimulation compared to younger dogs ($p<0.05$).

Conclusions: Aging in dogs is characterized by a decrease in lymphocyte subset counts and significant immunophenotypic changes. Further research is needed to understand the biological factors influencing aging and the development of age-associated inflammatory conditions in dogs.

Tuesday, November 19, 2024
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INVESTIGATING EFFECTS OF STORAGE ON CANINE PACKED RED BLOOD CELL CD47 EXPRESSION AND INTERACTIONS WITH MACROPHAGE-LIKE DH82 CELLS

Sydney Hastain, Alex Tufano, Sarah Musulin, Erika Gruber
North Carolina State University, Raleigh, NC, USA

Background: CD47 is a cell surface protein that inhibits phagocytosis by macrophages. CD47 is decreased in stored human red blood cells (RBCs), suspected to contribute to decreased RBC lifespan. Neither expression of CD47 nor susceptibility to macrophage binding and/or erythrophagocytosis have been investigated in canine RBCs.

Objective: Quantify surface expression of CD47 in stored canine packed RBCs and determine whether increased storage time increases susceptibility to RBC binding and/or erythrophagocytosis in vitro.

Methods: Packed RBCs from 5 healthy dogs were stored at 4°C and analyzed weekly for 7 weeks. Median fluorescence intensity (MFI) of CD47 surface expression was measured by flow cytometry on days 9, 16, 23, 30, 37, and 44. In parallel, RBCs were fluorescently labeled with carboxyfluorescein succinimidyl ester and incubated with canine DH82 macrophage-like cells. Fluorescence of DH82 cells was measured by flow cytometry.

Results: CD47 expression decreased by 45% (IQR: 33.6, 48.4) between days 9 and 44. DH82 cells incubated with labeled RBCs displayed a positive shift in fluorescence and small subpopulation of cells with higher fluorescence. The proportion of fluorescent positive DH82 cells was highest on day 37, accounting for approximately 39.5% of total events. The proportion of DH82 cells in the highly fluorescent subpopulation was largest on day 16, accounting for approximately 9.7% of all cells and 90.6% of fluorescent-positive cells.

Conclusions: CD47 expression decreases with storage of packed RBCs. Storage increases interactions between canine RBCs and DH82 cells. Additional studies are needed to determine whether this is due to increased binding or erythrophagocytosis.

Tuesday, November 19, 2024
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FELINE VENTRAL ABDOMEN LYMPHANGIOSARCOMA PRESENTING WITH HAEMOLYTIC ANAEMIA

Daniel McEvoy, Nutnapong Udomteerasuwat, Claire Andreasen
Iowa State University, Ames, IA, USA

An 11-year-old, male neutered, DSH was presented for a two-week history of regenerative anaemia and acute onset lethargy and ventral abdominal bruising. Haematology confirmed a marked macrocytic hypochromic regenerative anaemia, moderate thrombocytopaenia, moderate band neutrophilia and mild lymphopenia as well as hyperbilirubinemia and prolonged PT and aPTT. FeLV/FIV SNAP test and PCR for Cytauxzoon felis, Babesia, Apicomplexa and haemotropic Mycoplasma were also negative. Abdominal CT identified a poorly defined lobular structure in the inguinal area. Cytology of the area revealed atypical mesenchymal cells with erythrophagocytosis, haemorrhage and neutrophilic inflammation, with differentials including a sarcoma or histiocytic neoplasm. Euthanasia was elected due to poor prognosis and deteriorating condition. Necropsy was performed with histopathology of the region characterising plump spindle to stellate shaped neoplastic cells forming blood filled spaces, supported by collagen. The neoplastic cells were immunoreactive for Factor VIII, CD31, and PROX-1, confirming the neoplasm as a lymphangiosarcoma. This case report documents a rare occurrence of a feline ventral abdomen lymphangiosarcoma presenting with an ineffective regenerative chronic anaemia. The primary cause of the anaemia was not determined in this case; however, likely encompasses some aspect of paraneoplastic immune-mediated destruction, erythrophagocytosis by tumour cells, and haemorrhage. Although phagocytic IMHA was not confirmed, it is considered likely due to the marked hyperbilirubinemia and elimination of other causes of haemolytic anaemia. This case represents a

rapidly progressive lymphangiosarcoma (12 days from the initial veterinary visit) presenting with a marked anaemia.

Tuesday, November 19, 2024
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STANDARDIZED ASSESSMENT OF CANINE BONE MARROW CYTOLOGY AND HISTOLOGY SLIDES

Dorothee Bienzle[1], Kristina Meichner[2], Joanne Messick[3], Tracy Stokol[4]
[1]University of Guelph, Guelph, ON, Canada, [2]University of Georgia, Athens, GA, USA, [3]Purdue University, Lafayette, IN, USA, [4]Cornell University, Ithaca, NY, USA

Background: Bone marrow is a complex tissue composed of multiple cell types and subtly dissimilar cell differentiation stages. Reproducible assessment of alterations in the composition of hematopoietic, adipose and other tissue constituents is challenging, and in other species concurrent evaluation of cytology and histology preparations in a standardized manner has been proposed to maximize diagnostic return.

Hypothesis: A standardized approach for bone marrow assessment will lead to high concordance of morphologic findings and disease categorization between pathologists.

Methods: Study participation was invited via the ASVCP Listserv. The primary investigators derived and shared via webinar a comprehensive scheme with criteria for scoring and interpreting cytology and histology bone marrow slides. Responses for ~60 cytology and ~40 histology parameters, including mandatory and optional answers, were obtained. Eighteen paired cytology-histology glass slides with signalment and hematology results were mailed sequentially to 27 participants. Results were entered via a survey tool and compiled for analysis.

Results: There was >80% concordance for descriptive criteria of smear/section quality, hematopoietic cellularity, iron stores, megakaryocyte numbers, changes in stromal elements, infectious organism identification, and disease categorization. Granulocytic to erythrocytic cell ratios derived manually in cytology smears were more variable than categorical estimates for histology smears. While agreement on samples with acute leukemia was >90%, it was lower for presence and magnitude of myelodysplasia and immune reactivity.

Conclusions: Using the standardized approach, preliminary descriptive statistics indicate that there was high concordance for key elements and disease categories of bone marrow samples. Detailed statistical analysis of agreement is in progress.

Tuesday, November 19, 2024
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COMPARISON OF DIGITAL AND CONVENTIONAL PATHOLOGY METHODS FOR THE EVALUATION OF VETERINARY BLOOD FILMS

Stephanie Anderson[1], Trey DeJong[2], Mara Varvil[1]
[1]Washington State University, College of Veterinary Medicine, Veterinary Clinical Sciences, Pullman, WA, USA, [2]Washington State University, College of Arts and Sciences, Department of Mathematics and Statistics, Pullman, WA, USA

Background: Digital microscopy systems are used extensively in veterinary diagnostic pathology, but limited independent research has been published on its use, especially for the purpose of evaluating blood films. There is a need to determine potential limits of blood film assessments obtained via digital microscopy.

Objective: To compare the agreement of digital and glass cytology for the detection of common cellular morphology changes and abnormalities on veterinary blood films.

Methods: Veterinary clinical pathologists (15/22) and residents (7/22) evaluated canine (5/10), feline (3/10) and equine (2/10) blood films on glass and digital slides, with an 8+ week washout period between evaluations. Using a standardized rubric, sixteen erythrocyte features, two platelet features, and two leukocyte features were scored from absent to 4+. A leukocyte differential, including rubricytes, was performed. Additional comments at pathologist discretion were recorded.

Results: Erythrocyte shape changes, platelet changes and marked toxic change were readily identified on digital slides. Subtle toxic changes were frequently overlooked on digital slides, compared to glass slides. One case containing Piroplasm organisms, from a horse experimentally infected with *Theileria haneyi,* were identified on 50% of glass slides and 0% of digital slides.

Conclusions: Relative to glass slides, digital blood films appear adequate to identify most erythrocyte and platelet changes, and possibly inadequate to identify subtle leukocyte changes and intracellular infectious agents. Clinicians should exercise caution when interpreting results from digital blood film assessment.

Tuesday, November 19, 2024
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ESTABLISHMENT AND UTILIZATION OF CELL BLOCKS IN CANINE AND FELINE CAVITARY EFFUSION ANALYSIS

Kylie Pon, Bruce Wobeser, Melissa Meachem
Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Background: The cell block (CB) is a diagnostic technique of condensing cytologic material into a cellular pellet that is processed into a formalin-fixed paraffin-embedded (FFPE) histologic specimen for further analysis.

Objective: To evaluate diagnostic utility of CBs for cavitary effusion analysis in canine and feline patients.

Methods: CBs were created from canine and feline cavitary effusions (n=134) with various cytologic diagnoses using cell tube block and plasma-thrombin techniques. CB were assessed via cellularity, cell preservation, and histologic description. Immunohistochemistry (IHC) was performed as appropriate. Diagnostic certainty scores were assigned and compared between cytology interpretation alone and interpretation of cytology+CB+IHC combined.

Results: CBs were successfully made using both techniques, with adequate diagnostic cellularity in 80.6% (108/134). IHC was successfully performed using standard FFPE tissue protocols. Lymphocyte populations were characterized in 21 lymphocyte-rich effusions using CD3 and CD20. Cytokeratin, vimentin, and Wilms' tumor-1 were used to differentiate mesothelial cells from neoplastic epithelial cells in 26 atypical mesothelial/epithelial effusions. Feline coronavirus (FCoV) was identified within macrophages in 9/10 samples with a clinical diagnosis of feline infectious peritonitis (FIP); while FCoV was negative in 19 feline control cases with diagnoses other than FIP. In cases with confirmed neoplasia, assessment of CB increased detection rate of malignancy by 15.8% (8/38 to 14/38). Finally, additional information gained from CB increased diagnostic certainty of interpretation in 31.5% (34/108) of cases.

Conclusion: CBs provide additional diagnostic information in canine and feline cavitary effusion analysis and may be a useful ancillary test to perform in conjunction with conventional cytology.

Tuesday, November 19, 2024
11:45 AM – 12:00 PM

CYTOLOGY IS ESTIMATED TO BE HIGHLY SPECIFIC FOR PROCESSES COMMONLY IDENTIFIED ON FINE NEEDLE ASPIRATES OF CUTANEOUS AND SUBCUTANEOUS LESIONS

Helen Michael, Amy Valenciano, Laura Snyder, Corie Drake, Kimberly Yore, Heidi Peta
IDEXX Laboratories, Westbrook, ME, USA

Evaluating cytology performance, agreement between pathologists, and subjective confidence in cytologic interpretation is complicated. Pathologists vary in their comfort providing definitive diagnoses and in communication of confidence or uncertainty. We evaluated cytologic performance and inter-pathologist variability on common canine cutaneous and subcutaneous lesions. 837 digitally scanned cases were randomly assigned to three of six participating pathologists for evaluation. Pathologists were blinded to other results and used structured inputs to label processes and indicate a top differential. Five categories were included: poorly diagnostic/non-diagnostic (PD), normal fat/lipoma (fat), mast cell tumor (MCT), malignant neoplasia, and inflammation. To avoid biased performance estimates with majority consensus, an expectation maximization algorithm was used to estimate median pathologist sensitivity (Se), specificity (Sp), and 95% confidence intervals through bootstrap sampling of the data. There was high inter-pathologist variability for classifying inflammation as a top differential. Inter-pathologist variability decreased, and accuracy improved (Se: 0.809 [0.753, 0.874], Sp: 0.938 [0.913, 0.961]) for identification of any inflammation. Cytology was estimated to be moderately sensitive and highly specific for identifying top differential of: PD samples (Se: 0.829 [0.762, 0.890], Sp: 0.910 [0.882, 0.939]), fat (Se: 0.823 [0.735, 0.891], Sp: 0.990 [0.982, 0.997]), and malignant neoplasia (Se: 0.845 [0.770, 0.905], Sp: 0.976 [0.965, 0.986]), and highly sensitive and specific for identifying MCT as a top differential (Se: 0.929 [0.864, 0.983], Sp: 0.999 [0.995, 1.00]). Individual pathologist varied most in identifying processes as “primary.” In this study, cytology had high estimated specificity for identification of the top differential in cutaneous and subcutaneous lesions.

Diagnostic Pathology Abstracts

Chair: Francisco Uzal

Sunday, November 17, 2024
01:30 PM – 01:38 PM

H5N1 CLADE 2.3.4.4B VIRUS INFECTION AMONG GOAT KIDS

Ariel De Aguiar[1], George Kaslow[1], Erica Wietgrefe[1], Mackenzie Reberg[2], Mia Torchetti[3], Jennifer Johnson[3], Kimberly Lehman[3], Rebecca Cox[3], Erik Olson[1]
[1]University of Minnesota, Saint Paul, MN, USA, [2]USDA - APHIS, Saint Paul, MN, USA, [3]USDA-APHIS-VS-D&B-NVSL-DBPL, Ames, IA, USA

Background: The highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b has led to outbreaks among U.S. commercial poultry and backyard flocks since 2022, with documented spillover to various mammal species. In March 2024, several newly kidded farmed goats developed acute neurological signs prior to death. The goat herd shared the pasture and water source with a flock of chickens and ducks, which had been recently depopulated due to HPAI.

Methods and Results: A goat kid from this herd was necropsied with no significant macroscopic abnormalities. Histologic lesions involved cerebrocortical neuroparenchymal vacuolation, gliosis, neuronal degeneration/necrosis, edema, and neutrophilic and lymphoplasmacytic meningoencephalitis. The heart exhibited mild cardiomyocyte necrosis with pleocellular myocarditis. Intralesional influenza A antigen was detected immunohistochemically in the brain and heart. Frozen tissues were tested by matrix and H5 clade 2.3.4.4b-target PCR assays with detections in multiple organs. Whole genome sequencing and phylogenetic analysis revealed the virus as HPAI H5N1

clade 2.3.4.4b genotype B3.6 with high identity to the virus from the recently depopulated ducks and chickens. Genotype B3.6 replaced genotype B3.2 as the predominant genotype circulating in wild birds during the fall of 2023. Subsequent PCR testing of nine additional mortalities identified four kids with the HPAI virus (as well as concurrent *Coxiella burnetti*); adult nannies tested negative.

Conclusions: This is the first reported natural infection of a ruminant by HPAI H5N1 in North America. The farm's history of HPAI in poultry and the shared environment between the birds and goats likely contributed to cross-species transmission.

Sunday, November 17, 2024
01:38 PM – 01:46 PM

FELINE CHRONIC BILIARY OBSTRUCTION SECONDARY TO GASTROINTESTINAL EOSINOPHILIC SCLEROSING FIBROPLASIA

William Holl, Fabio Del Piero
Louisiana State University, Baton Rouge, LA, USA

History: A 2.5-year-old, female, domestic longhair cat was icteric secondary to suspected liver failure and had an intraabdominal mass near the coastal arch. The cat was not gaining weight and was brought to a veterinarian where it suddenly died and was submitted for necropsy.

Description: The bile duct was not patent and firm towards the papillary orifice. The gallbladder was bilobed and distended with a thickened wall and multiple cystic dilatations containing abundant clear yellow and white opaque, viscous to gelatinous bile. The liver was mottled tan and purple with an accentuated lobular pattern, firm, and enlarged weighing 142 g (5.26% of the body weight). Many bile ducts were diffusely ectatic. The intestinal wall at the duodenal papilla was firm, thickened, and regionally ulcerated. Microscopically, the gallbladder was expanded by fibrosis, and the liver has extensive portal bridging fibrosis with an intense ductular reaction and many mixed inflammatory infiltrates as well as congestion and hepatocellular atrophy which intensified towards larger bile ducts. The intestinal wall was effaced by anastomosing bundles of dense collagen and fibroplasia with many infiltrating eosinophils.

Summary: This is a case of chronic extrahepatic biliary obstruction (EHBO) secondary to gastrointestinal eosinophilic sclerosing fibroplasia in a cat (FGESF). Feline EHBO is uncommon and associated with various conditions. FGESF is a focal to multifocal eosinophilic inflammatory response with an unknown pathogenesis resulting in firm intramural masses historically associated with regions near the pylorus or ileocecocolic junction although recent research has demonstrated higher prevalence in the proximal duodenum.

Sunday, November 17, 2024
01:46 PM – 01:54 PM

PATHOLOGY AND LOCALIZATION OF AVIAN REOVIRUS IN POULTRY WITH VIRAL TENOSYNOVITIS

Cheng-Shun Hsueh, Pablo Piñeyro, Olufemi Fasina, Mohamed Medhat El-Gazzar, Yuko Sato
Iowa State University, Ames, IA, USA

The re-emergence of Avian reovirus (ARV) is associated with various conditions, including arthritis/tenosynovitis, hepatitis, myocarditis, encephalitis, and enteritis. Despite extensive research, comprehensive histopathologic and viral antigen distribution of ARV-associated tenosynovitis are scarce. This study elucidates the pathologic features of ARV tenosynovitis, coupled with in situ hybridization (RNAscope) to localize ARV M1 transcripts in lesions. Inclusion criteria for this study were legs from two chicken flocks (5-10 weeks old; 37 legs) and two turkey flocks (17-30 weeks old; 12 legs) submitted to ISU-VDL for necropsy with a clinical history of lameness, positive ARV qRT-

PCR results (ranging from 21.7-26.5), and histologic lesions consistent with ARV. Gross findings included serous-to-viscous fluid within swollen joints in all flocks (4/4, 100%); affecting 18/37 (48.6%) chicken legs and 6/12 (50%) turkey legs. Hemorrhagic deep digital flexor and gastrocnemius tendons were seen in both chicken flocks (9/37; 24.3% of legs). Histologically, tenosynovitis (4/4, 100%) was characterized by predominant infiltrates of lymphocytes, plasma cells and macrophages in the synovial intima and subintimal fibrous tissues, and frequent perivascular lymphocytic nodules (3/4, 75%). The subintima stroma was usually expanded by fibrous tissue with neovascularization (3/4, 75%). The synovial membrane was thickened and papillated by hyperplastic synoviocytes (4/4, 100%). RNAScope revealed viral transcripts in the synovial subintimal fibroblasts of affected synovium (4/4, 100%). Other infectious causes were ruled out by histopathology, bacterial culture, and Mycoplasma PCR. This study provides the first report highlighting the detection of ARV transcripts within subintimal fibroblasts in lesions, offering insights into ARV diagnostics and pathogenesis.

Sunday, November 17, 2024
01:54 PM – 02:02 PM

HAIRY VETCH TOXICOSIS IN A CATTLE HERD

Hilary Ann Lakin[1,2,3], Viju Pillai[1,3], Mario Sola[1,3], Gene Niles[4], Stephen Hooser[1,3]
[1]Purdue University College of Veterinary Medicine, West Lafayette, IN, USA, [2]National Institutes of Health CBSTP, Bethesda, MD, USA, [3]Indiana Animal Disease Diagnostic Laboratory, West Lafayette, IN, USA, [4]Colorado State University College of Veterinary Medicine, Fort Collins, CO, USA

Abstract: An outbreak of severe nodular to diffuse dermatitis in black yearling cattle occurred in early summer on a farm in northeast Colorado, resulting in the mortality of nineteen cattle. The survivors exhibited symptoms of anorexia, rough scaly skin, and chronic weight loss. Clinical evaluation revealed poor body condition, hyperthermia, pruritus, and superficial lymphadenopathy. Notably, the affected cattle were restricted to grazing on abandoned farmland, and symptomatic treatments provided were ineffective. Postmortem examination revealed marked lymphadenopathy and multifocal pale lesions on the cut surfaces of the skin, kidneys, spleen, and liver. Histopathological analysis demonstrated severe, nodular to diffuse granulomatous dermatitis characterized by eosinophils and multinucleated giant cells. Similar eosinophilic granulomatous inflammation was noted in the kidneys, liver, and spleen. These findings are consistent with hairy vetch (Vicia villosa) toxicosis, as the cattle had been grazing on pastureland containing hairy vetch.

Sunday, November 17, 2024
02:02 PM – 02:10 PM

DIAGNOSTIC INVESTIGATION AND ISOLATION OF UREAPLASMA DIVERSUM ASSOCIATED WITH REPRODUCTIVE DISORDERS IN CATTLE

Calvin Ko[1], Alyona Michael[2], Rachel Derscheid[1], Ganwu Li[1], Christopher Siepker[1]
[1]Iowa State University, Ames, IA, USA, [2]Auburn University, Auburn, AL, USA

Ureaplasma diversum is a fastidious bacterium that is associated with bovine granular vulvovaginitis, mastitis, placentitis, and fetal pneumonia. As a commensal organism, diagnosis of *U. diversum* can be challenging. There has yet to be a comparison of modern culture media to grow *U. diversum,* and protocols vary among laboratories. This study investigates three clinical cases with histopathology, *in situ* hybridization (RNAscope®), molecular diagnostics, a comparative *Ureaplasma* culture in four media, and sequencing analysis. Histopathology demonstrated necrosuppurative placentitis in all three cases, fetuses from two cases also had suppurative to lymphocytic bronchopneumonia. RNAscope® highlighted *U. diversum* in the chorioallantoic villi and stroma in two cases. *U. diversum* qPCR were positive in all three cases in lung, stomach content, and/or placenta and PCR negative for Bovine Herpesvirus-1, Bovine Viral Diarrhea Virus, *Leptospira* spp., *Neospora caninum*, and *Mycoplasma bovis*. Routine and *Campylobacter* culture were negative. *Ureaplasma* culture was

attempted on four liquid media: SP4 and *Ureaplasma* broth (commercial), and Hayflick and Mycoplasma Experience (ME) broth with 0.05% urea, antibiotics, at pH 6.0. *U. diversum* was successfully isolated from two cases through SP4 and ME broth. Genetic sequencing analysis of *Ureaplasma* yields a phylogenetic tree and virulence factor analysis comparing these pathogenic isolates, reference strains, and strains from clinically normal herds. This diagnostic investigation demonstrated that *U. diversum* is a potentially underdiagnosed bovine abortifacient agent with possible misdiagnosis due to its commensal nature. Microscopic lesions, *in situ* characterization, PCR detection, and proper *Ureaplasma* culture method are useful diagnostic tools in challenging scenarios.

Sunday, November 17, 2024
02:10 PM – 02:18 PM

NINE CASES OF PRESUMED PRIMARY CAUDAL ABDOMINAL HEMANGIOSARCOMA ASSOCIATED WITH THE URINARY BLADDER SEROSA IN DOGS

Maya Schlesinger, Devinn Sinnott, Kevin Woolard
University of California, Davis - School of Veterinary Medicine, Davis, CA, USA

Hemangiosarcoma (HSA) is a commonly diagnosed neoplasm in dogs with important clinical and prognostic implications. HSA arising from the serosa of the urinary bladder and/or soft tissues of the pelvic inlet is poorly described as a primary tumor location and is considered a subset of the overall relatively uncommon retroperitoneal hemangiosarcoma (RPHSA). Nine cases of hemangiosarcoma associated with the urinary bladder serosa or pelvic inlet were identified in necropsy (n=8) and biopsy (n=1) submissions at the University of California, Davis - Veterinary Medical Teaching Hospital (VMTH) between 2003 and 2023. Clinical data and histopathology for these cases were reviewed. Primary presenting complaints included hemoabdomen (n=2), difficulty or changes in urination (n=4), cutaneous bleeding masses (n=1) or other clinical signs unrelated to the abdominal HSA (cerebral disease, n=1; cardiac abnormalities and lethargy, n=1). Patients ranged from 6.3 to 14.1-years-old at the time of diagnosis (mean=10.6-years-old), represented 8 different breeds, and were distributed evenly across sex (male-castrated, n=5; female-spayed, n=4). Grossly, the masses were predominantly dark red to tan, variably cavitated, and associated with hematoma formation. Histologically, these neoplasms were often closely associated with the urinary bladder serosa and neoplastic mesenchymal cells formed cavernous channels filled with hemorrhage and fibrin, characteristic of HSA. These findings demonstrate that the urinary bladder serosa and pelvic inlet are clinically important sites for HSA occurrence and should be considered as a differential diagnosis for dogs presenting with hemorrhagic caudal abdominal masses and urinary dysfunction.

Sunday, November 17, 2024
02:18 PM – 02:26 PM

HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL REVIEW OF EASTERN EQUINE ENCEPHALITIS VIRUS MULTI-SPECIES OUTBREAK IN CONNECTICUT, 2023

Arashi Nakashima, Neha Mishra, Emily Reinhardt, Kerr Kirklyn
University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Storrs, CT, USA

Background: Eastern Equine Encephalitis virus (EEEV), part of the Togaviridae family and Alphavirus genus, is transmitted between mosquitoes and avian reservoir hosts, occasionally affecting incidental hosts like humans and horses. Periodic outbreaks, including one in Connecticut in 2023, highlight its public health significance.

Objective: This study presents the histopathologic and immunohistochemical findings from seven diagnosed cases of EEEV in Connecticut during the reporting year of 2023. The cases include two equines, two ring-necked pheasants, two ravens, and one emu.

Methods: Cases were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) for postmortem examination. Five cases exhibited neurological symptoms and sudden death. Tissue sections from various organs were stained with Hematoxylin and Eosin (H&E). Brain samples were tested for EEEV and West Nile Virus (WNV) via PCR. EEEV immunohistochemistry (IHC) was performed at the Louisiana Animal Disease Diagnostic Laboratory (LADDL).

Results: All cases tested positive for EEEV and negative for WNV by PCR. Histopathologic examination revealed perivascular encephalitis in the horses and pheasants, while the emu and ravens showed neurodegenerative changes with minimal inflammation. IHC revealed strong immunopositivity in neurons and glial cells of the horses and pheasants, whereas the emu and ravens exhibited rare immunopositivity.

Conclusions: This study confirmed EEEV infection in multiple avian and aberrant mammalian hosts. Further this work highlights the comparative neuropathology and immunohistochemical staining seen in different host species.

Sunday, November 17, 2024
02:26 PM – 02:34 PM

CORRELATION OF PCV2 QUANTIFICATION ON TISSUES BY QPCR, IMMUNOHISTOCHEMISTRY, AND IN-SITU HYBRIDIZATION IMAGE DIGITAL ANALYSIS

Trevor Arunsiripate, Molly Kroeger, Rodrigo de Paiva, Pablo Pineyro
Iowa State University College of Veterinary Medicine Department of Veterinary Diagnostic and Production Animal Medicine, Ames, IA, USA

Porcine circovirus type 2 (PCV2) is responsible for porcine circovirus-associated disease (PCVAD). The disease diagnosis criteria include presence of clinical signs, characteristic histological lesions, and confirmation of viral replication. PCV2 replicates within lymphocytes and macrophages, causing lymphoid depletion and inflammation. While commercial vaccines reduce viremia and lesions, they do not abrogate viral replication. Detection is confirmed by PCR; but PCR results alone cannot correlate with disease severity, lesions or viral replication, limiting its diagnostic utility in disease confirmation. This study aims to correlate PCV2 PCR Ct values with PCV2 mRNA levels detected by *in-situ* hybridization (ISH) and antigen by immunohistochemistry (IHC) in vaccinated and unvaccinated experimentally challenged animals. Eighty pigs were vaccinated at 21 days of age with a commercial PCV2 vaccine while 20 remained unvaccinated. All pigs were inoculated with 1 mL IM and 1 mL IN of PCV2d (5 $\log_{10}$/2ml dose) at 49 days-of-age. All pigs were euthanized 28 days post challenge and tissues were collected (tonsil, lung, and lymph nodes) for ISH, IHC and qPCR. Then, both the ISH and IHC signal was quantified by digital analysis using HALO. In unvaccinated pigs, positive correlation was observed between ISH/IHC signal and PCR Ct values. Vaccinated pigs showed positive qPCR in all tissues, but no quantifiable ISH or IHC signal. These findings suggest that vaccination reduces viral replication detectable by direct method and PCR alone may not be sufficient to confirm PCVAD. Direct and digital quantitative detection of PCV2 IHC or ISH can be used to confirm PCVAD.

Sunday, November 17, 2024
02:34 PM – 02:42 PM

AMOEBIC ENTERITIS AND HEPATOCELLULAR NECROSIS IN AN ADULT PANTHER CHAMELEON (FURCIFER PARDALIS) – A CASE REPORT

Alexandra Reddy, Vanessa Oakes, Michelle Todd, Thomas Cecere, Kevin Lahmers, Stephen Smith
Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

An adult, intact-male panther chameleon (*Furcifer pardalis*) was humanely euthanized following a one-week history of bloody urate production, lethargy, and anorexia. Gross necropsy evaluation

revealed diffuse intestinal dilatation and transmural dark red-brown to green-tinged discoloration, caseous intestinal contents, and multifocal, pinpoint, white foci throughout all liver lobes. Histopathological analysis of formalin-fixed samples of intestinal and hepatic tissue demonstrated heterophilic and fibrinonecrotic ulcerative enteritis, as well as hepatocellular degeneration and necrosis, with intralesional unicellular trophozoites measuring up to 17 um in diameter consistent with the genus *Entamoeba.* Transmission electron microscopic evaluation of formalin-fixed intestinal samples highlighted ultrastructural features of trophozoites consistent with *Entamoeba*, including pseudopod formation, central nuclei with peripheralized chromatin, and the presence of chromatoid bodies within the cytoplasm. Herein, we report the first recognized case of amoebic enteritis and hepatocellular necrosis in a panther chameleon (*Furcifer pardalis*) to our knowledge. Although one of the most clinically significant diseases of reptiles, pathogenic amoebiasis is rarely reported in lizards, yet it is of critical concern in zoological institutions due to its high mortality in susceptible species such as lizards. Therefore, this case report demonstrates the importance of clinical recognition and histologic diagnosis of amoebiasis in reptile species to enhance their health and welfare under managed care.

Sunday, November 17, 2024
02:42 PM – 02:50 PM

NODAL PLEXIFORM VASCULOPATHY IN 2 CATS: HISTOLOGICAL PRESENTATION AND IMMUNOHISTOCHEMICAL DISTRIBUTION OF VASCULAR MARKERS

Marvin Firth, Sandra Dawson, Malcolm Silkstone
Nationwide Laboratories, Poulton-Le-Fylde, United Kingdom

Two feline lymph node submissions to a UK diagnostic laboratory were found to have histological features consistent with nodal plexiform vasculopathy.

Plexiform vasculopathy, (syn. vascular transformation of lymph nodes), is an endothelial proliferation within lymph nodes and has been reported in humans [1], cats [3-7], and one dog with thyroidal carcinoma[2]. It is an uncommon lesion, and it is still unknown whether the proliferating endothelial cells are of lymphatic or vascular origin with recent reports proposing a lymphendothelial origin[3]. The literature describes it as a lymphadenopathy with vasoproliferation and lymphoid atrophy (as seen histologically in both cases). The lesions are usually focal but can be extensive; often mimicking hemangiosarcoma. The pathogenesis of this disease is unclear in both the human and animal literature. The description has been limited to cats and one dog with no large numbers studied. Plexiform vasculopathy is most documented in the cervical lymph nodes of cats, with one recent report in a serosal lymph node[6] and one in a retropharyngeal lymph node[7], and has been associated with malignant transformation to angiosarcoma[5].

This report highlights histological features consistent with nodal plexiform vasculopathy within novel anatomical locations (prescapular and mammary lymph node) in two individuals, which to the authors' knowledge has not yet been reported. Utilising immunohistochemical markers for vascular and lymphatic vessels, distribution of potential histogenesis is demonstrated. Nodal plexiform vasculopathy, whilst reported to be a rare condition, should be considered by diagnostic pathologists when performing histopathology of the lymph node with a clinical history of lymphadenopathy.

Sunday, November 17, 2024
02:50 PM – 02:58 PM

EPIDEMIOLOGY AND PATHOLOGY OF YERSINIOSIS-INDUCED ABORTION IN SMALL RUMINANTS: A 22-YEAR CASE SERIES STUDY AT THE CALIFORNIA ANIMAL HEALTH AND FOOD SAFETY LABORATORY SYSTEM: 2002-2023

Seunghee Cho[1], Asli Mete[2], Isaiah Cueva[2], Melissa Macías-Rioseco[3], Heather Fritz[2], Nicolas Streitenberger[2], Omar Gonzales-Viera[2]

[1]Department of Comparative Pathobiology/ADDL, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA, [2]California Animal Health and Food Safety Laboratory system (CAHFS), Davis Laboratory, University of California, Davis, Davis, CA, USA, [3]California Animal Health and Food Safety Laboratory system (CAHFS), Tulare Laboratory, University of California, Davis, Tulare, CA, USA

Background

Abortion in small ruminants poses a significant economic threat and might have zoonotic causes. While the association between yersiniosis and reproductive complications is known, systematic studies and case series on abortion in sheep and goats are scarce.

Objective

This study aims to characterize the epidemiological and pathological features of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*-induced abortions in small ruminants, contributing to the understanding of this zoonotic disease in California.

Methods

A 22-year retrospective study was conducted to examine microbiological and pathological findings in submissions for abortion diagnostics, as well as the geographic and seasonal distribution of disease.

Results

Yersiniosis-induced abortion was diagnosed in 22 goats and 12 sheep, with all abortions occurring in the 3rd trimester. Samples from lung, liver, placenta, and abomasal contents were submitted for aerobic culture, and abomasal contents showed the highest recovery of *Yersinia* spp. Microscopically, there was severe necrotizing and suppurative inflammation in the lung, liver, spleen, kidney, and, when present, the placenta. There was evidence of hepatic copper, zinc, and selenium deficiency in nine, two, and three cases, respectively. Geographically, cases were concentrated in Northern and Central California, with a seasonal pattern favoring winter and spring occurrences.

Conclusions

In conclusion, this 22-year retrospective study at the California Animal Health and Food Safety Laboratory System significantly contributes to the understanding of the epidemiological and pathological features of *Yersinia spp.*-induced abortions in small ruminants.

Sunday, November 17, 2024
03:30 PM – 03:38 PM

KLEBSIELLA PNEUMONIAE INFECTION IN RACCOONS (PROCYON LOTOR): REPORT OF THREE CASES

Matías Dorsch[1], William Holl[1], Bianca de Cecco[1], Laura Peak[2], Pablo Fresia[3], Christine Walsh[2], Maria Mitchell[2], Marge Gill[1], Cesar Menk[1], Emi Sasaki[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]Louisiana Animal Disease Diagnostic Lab, Baton Rouge, LA, USA, [3]Unidad Mixta Pasteur + INIA, Institut Pasteur de Montevideo, Montevideo, Uruguay

Background: *Klebsiella pneumoniae* is a gram-negative bacterium associated with opportunistic respiratory, reproductive, and urinary infections, that may lead to fatal septicemia.

Objective: This study describes the gross, histologic, microbiological, and molecular features of three cases of klebsiellosis in raccoons (*Procyon lotor*).

Methods: Gross and histopathological examination, bacterial culture, and whole genome sequencing (WGS) were performed.

Results: Three 5-to-7-month-old raccoons died after exhibiting lethargy, anorexia, tachypnea, and diarrhea. Grossly, the peritoneal cavity contained up to 600 mL of opaque, white to yellow, viscous material (3/3). The omentum was diffusely dark red. Mesenteric lymph nodes were diffusely enlarged, and white viscous fluid oozed from the cut surface (2/3). Microscopically, the omentum and serosal surfaces of abdominal viscera were markedly infiltrated by neutrophils and foamy macrophages occasionally containing short, encapsulated, gram-negative rods, mixed with fibrin and necrotic debris. In the mesenteric lymph nodes, there was pyogranulomatous lymphadenitis with areas of necrosis, and numerous intracellular and extracellular gram-negative short rods. Other findings included ulcerative ileotyphlitis with intralesional bacteria (1/3), eosinophilic enterocolitis with intraluminal parasites (2/3), neutrophilic and histiocytic splenitis (2/3), and eosinophilic adrenalitis (1/3). Hypermucoviscous *Klebsiella pneumoniae* was isolated in pure culture from peritoneal swabs and lymph nodes (3/3). WGS from one isolate determined the strain type (ST60) and the presence of well-known virulence factors including capsule polysaccharide (*rmpA*), yersiniabactin (*ybt, irp1, irp2, fyuA*), and salmochelin (*iroB/C/D/N*).

Conclusions: Herein described is a fatal septicemic infection by *K. pneumoniae* in raccoons that, to our knowledge, has not been reported thus far.

Sunday, November 17, 2024
03:38 PM – 03:46 PM

PSEUDORABIES IN A HUNTING DOG

Matías Dorsch, Cesar Menk
Louisiana State University, Baton Rouge, LA, USA

Background: Domestic and wild swine are the natural hosts of Suid Herpesvirus-1 (SuHV-1), the causative agent of pseudorabies. Clinical disease in swine consists mainly of reproductive and respiratory signs, although wild hogs are mostly asymptomatic. Other species, including dogs, are accidental hosts that experience acute fulminant disease.

Objective: To describe the gross, histologic, and molecular features of a pseudorabies case in a dog.

Methods: Routine gross examination, histologic processing, and PCR for SuHV-1 were performed.

Results: A 3-year-old, female Plott hound canine died with a history of facial pruritus for a week following contact with a feral hog. Grossly, there were coalescing areas of cutaneous alopecia with subcutaneous hemorrhage and edema on the left frontal, temporal and maxillary regions. The tonsils were mildly enlarged. The lungs were congested with multifocal hemorrhages, and there was marked dirofilariasis in the heart. Microscopically, the trigeminal ganglion was infiltrated by lymphocytes and histiocytes. In the brainstem, glial nodules and lymphocytes were in the neuropil, and blood vessels were cuffed by lymphocytes and histiocytes. Multiple neurons in the trigeminal ganglion and brainstem were degenerated or necrotic, with rare satellitosis and neuronophagia. Affected neurons occasionally contained intranuclear glassy eosinophilic viral inclusions that peripheralized the chromatin. There were reactive lymphoid follicles with lymphocytolysis in the tonsils. SuHV-1 was detected by PCR in the brainstem.

Conclusions: Although pseudorabies has been eradicated in domestic pigs in the USA, the virus still circulates in wild boars across the country, thus posing significant threat to accidental hosts and commercial swine farms.

Sunday, November 17, 2024
03:46 PM – 03:54 PM

CATASTROPHIC DISTAL SESAMOIDEAN LIGAMENT RUPTURE IN RACEHORSES: A RETROSPECTIVE STUDY

Carlos Schild[1], Hernando Acevedo[1], Laura Kenedy[2], Jeff Blea[3], Akinyi Nyaoke[1], Javier Asin[1], Eileen Henderson[1], Susan Stover[4], Francisco Uzal[1]
[1]California Animal Health and Food Safety Laboratory System, University of California-Davis, San Bernardino, CA, USA, [2]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY, USA, [3]California Horse Racing Board, Arcadia, CA, USA, [4]Veterinary Orthopedic Research Laboratory, University of California-Davis, Davis, CA, USA

Complete rupture of the distal sesamoidean ligaments (DSLs) results in suspensory apparatus (SA) failure. We describe the pathology of a series of cases of DSL rupture in racehorses from California and Kentucky. Necropsy findings of all racehorses submitted for postmortem examination between 1992-2022 that had a DSL rupture (DSLR) were reviewed. Horse signalment, exercise circumstances, DSLs affected, the presence of gross pre-existing lesions of the DSLs (chronic desmitis) and other lesions of the SA (osteochondral fragmentations of the proximal sesamoid bones and/or chronic desmitis of the suspensory ligament) were analyzed by multiple correspondence analyses. Associations between lesions and all the other variables were compared by Fisher's exact test. Seventy-seven racehorses (87.0% Thoroughbred and 13.0% Quarter Horses), most 4-8-year-old (*median:* 5, *range:* 2-15), female (32.5%), gelding (28.6%) and intact male (38.9%) had a catastrophic DSLR (97.4% unilateral [60% left and 40% right]; 2.6% bilateral) while racing (88.0%) or training (12.0%). The DSL were completely (93.8%) and incompletely (6.2%) disrupted either in a transverse (84.2%) or longitudinal (15.8%) configuration. DSLR involved the cruciate (65.3%), oblique (97.3%), and straight (96.0%) DSLs. Only two (2/77, 2.6%) horses had an observed pre-existing lesion in the DSLs, whereas 21 (21/77; 27.3%) had other lesions of the SA. There was no associations between pre-existing gross lesions of the DSLs (P>0.10) or other lesions of the SA (P>0.10) and other variables. It is possible, however, that microscopic predisposing lesions were present in horses with DSLR, but were not visibly grossly. Further studies of DSLR, including histology, are needed

Sunday, November 17, 2024
03:54 PM – 04:02 PM

HISTOPATHOLOGIC CHANGES OF THE GALLBLADDER IN DOGS WITH EMPHYSEMATOUS CHOLECYSTITIS

Maya Schlesinger[1], Tarini Ullal[2], April Choi[1]
[1]University of California, Davis - Veterinary Medical Teaching Hospital, Anatomic Pathology, Davis, CA, USA, [2]University of California, Davis - Veterinary Medical Teaching Hospital, Small Animal Internal Medicine, Davis, CA, USA

Emphysematous cholecystitis (EC) is a relatively rare but important clinical diagnosis in dogs that is made by identification of gas in the wall and/or lumen of the gallbladder by computed tomography or ultrasound. EC has been associated with bacterial infection but neither a pathogenesis nor a distinct etiologic agent has been identified in veterinary medicine. Between 1999 and 2023, 19 dogs were diagnosed by imaging data with emphysematous cholecystitis at the University of California, Davis Veterinary Medical Teaching Hospital (VMTH). Of these, 7 patients had a cholecystectomy performed and their gallbladder submitted for histopathology. Clinical data, including aerobic and anaerobic culture of bile and/or liver tissue, and histologic sections of gallbladders were reviewed. There were

two distinct morphologic patterns in the submitted gallbladders: (1) mural necrosis with neutrophilic inflammation (n=4) or (2) mucosal cystic or papillary hyperplasia with lymphoplasmacytic inflammation (n=3) and there was no evidence of gas or emphysema in any case. Four cases had polymicrobial infections, two cases had monomicrobial infections, and one case had no growth. Bacteria included Clostridium perfringens (n=5), non-hemolytic Escherichia coli (n=3), Klebsiella pneumoniae (n=1), Streptococcus viridans (n=1), S. bovis-equinus complex (n=1), and Enterococcus faecium (n=1). Bacteria were visible by H&E staining in a majority of cases (n=5). In conclusion, despite a consistent clinical and ultrasonographic presentation, the histologic features and bacterial cultures vary and there is no histologic evidence of emphysematous change in clinical cases of EC.

Sunday, November 17, 2024
04:02 PM – 04:10 PM

NEOPLASIA IN PSITTACINES IN LOUISIANA: A 5-YEAR RETROSPECTIVE STUDY

Luis Tondo, Cesar Menk
Louisiana State University, Baton Rouge, LA, USA

Background: Neoplasms are commonly diagnosed in psittacines and their incidence increases with age, correlating with the longer life span of these animals. Although common, there are few reports on the most common neoplasms affecting psittacine birds.

Objective: Report the most commonly diagnosed neoplasms in psittacines over five years at the Louisiana Animal Diagnostic Laboratory (LADDL).

Methods: Reports from 2019 to 2024 at LADDL were retrospectively evaluated. Selected cases were revisited for significant morphological features.

Results: From 2019-2024, 293 psittacine birds were submitted for postmortem evaluation at LADDL; of these, 26 birds (8.87%) were affected by neoplasia. Of those birds, 13 were males, 12 were females, and one was of unknown sex. The age of affected birds ranged from 3 to 50 years. Malignant tumors were diagnosed in 25 birds, including 18 carcinomas, 5 sarcomas, and 1 round cell tumor. Carcinomas were most frequently observed in the gastrointestinal tract (8 cases), pancreas (2 cases), and thyroid glands (2 cases). Sarcomas were found in the coelomic cavity (3 cases) and spleen (1 case). The only round cell tumor in this period was a leukemia. The neoplastic phenotype could not be identified in one coelomic mass; and an adenoma was identified in the thyroid gland in one bird.

Conclusions: In the span of five years, 26/293 psittacines were diagnosed with at least one neoplasm. Malignant tumors were more common than benign neoplasms, with the gastrointestinal tract being the most commonly affected location for carcinomas, the most frequent malignant neoplasm in the studied animals.

Sunday, November 17, 2024
04:10 PM – 04:18 PM

WHITE SPOT SYNDROME VIRUS IN A COHORT OF IMPORTED BLUE CRAYFISH (PROCAMBARUS ALLENI)

Naomi Falconnier[1], Shannon Gray[2], Kuttichantran Subramaniam[2], Adrien Izquierdo[3], Nanny Wenzlow[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]University of Florida, Gainesville, FL, USA, [3]Audubon Nature Institute, New Orleans, LA, USA

Background: White spot syndrome virus (WSSV) is a globally important disease of crustaceans, causing economically devastating mortality in penaeid shrimp.

Objective: The goal of this study was to describe the histologic characteristics of a WSSV outbreak in a cohort of blue crayfish (*Procambarus alleni*) and molecularly characterize the viral strain.

Methods: Twenty blue crayfish were purchased from a New York vendor that were imported from Thailand. During quarantine, sixteen crayfish were found dead, five of which were submitted for postmortem examination, and one was frozen at -80°C. The remaining surviving four crayfish were subsequently humanely euthanized and also submitted for examination. All fixed crayfish were processed and stained routinely. Pooled samples of fresh-frozen cuticular epithelium, gills, and stomach were submitted for WSSV PCR and for Illumina Novaseq next-generation sequencing (NGS) and phylogenetic analysis.

Results: Histologic examination revealed large intranuclear viral inclusions, mainly within the cuticular and gastrointestinal epithelium, gills, and antennal glands of all naturally deceased crayfish and two euthanized crayfish. Those without viral inclusions had mild hemocyte infiltration of similar tissues. Initial WSSV PCR detected viral nucleic acid, and NGS generated a genetically distinct 281,205 bp WSSV genome that formed a unique branch among Australian and Asian prawn and shrimp isolates.

Conclusions: This is the first report of WSSV in blue crayfish, and the genetically distinct viral isolate clustered with Australian and Asian isolates, suggesting infection prior to shipment. This case illustrates potential disease spread through animal transportation. Increased awareness of imported infectious diseases could inform future governmental regulations.

Sunday, November 17, 2024
04:18 PM – 04:26 PM

HEREDITARY ATAXIA SYNDROME IN A DOG

Bianca de Cecco[1,2], Jocelyn Lee[1], Kylie Landry[1], Patty Lathan[1], Jeongha Lee[1,2]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]LSU-LADDL, Baton Rouge, LA, USA

A 1-year and 11-month-old male castrated Collie dog presented with worsening signs of hindlimb weakness and loss of appetite. Owners reported that the dog's littermates had similar neurologic signs. At examination, progressive para/tetraparesis, limited vision, and pale mucous membranes were observed. A clinical diagnosis of severe non-regenerative anemia was made, and due to poor prognosis, the dog was euthanized and submitted for postmortem examination. Grossly, there was severe pallor of the entire body. Microscopically, the lesions were observed in the central nervous system and concentrated in the cerebellum and spinocerebellar tracts and included extensive loss of granular cell layer and Purkinje cells in the cerebellum, and areas of gliosis and spongiosis in the cerebellar, cerebral, spinal cord white matter with prominent reactive astrocytes. Ocular lesions were bilateral and included retinal dysplasia and optic nerve degeneration. Bone marrow examination was supportive of precursor-targeted immune-mediated anemia. The association of progressive para/tetraparesis with spinocerebellar tract degeneration is highly suggestive of an inherited/genetic degenerative disorder similarly described in humans and categorized as spinocerebellar ataxia which falls under the umbrella term "hereditary ataxia". Hereditary ataxias are a heterogeneous group of neurodegenerative diseases characterized clinically by cerebellar or spinocerebellar dysfunction with ataxia as the main clinical sign. These diseases appear to be caused by genetic mutations/defects interfering with basic cellular functions such as autophagy and degradation, cation trafficking, or transport. In our case, the observed lesions in the central nervous system, and ocular structures classify this hereditary ataxia as multifocal degeneration with predominant spinocerebellar component.

Sunday, November 17, 2024
04:26 PM – 04:34 PM

MALAKOPLAKIA IN A YOUNG CAT

Sophie Hopkins, Ryan Jennings
Ohio State University, Columbus, OH, USA

Malakoplakia is an uncommon inflammatory condition that most often affects the urogenital tract of immunocompromised individuals. A stray young adult female domestic shorthair cat presented with dehydration, a body condition of 1/9, and firm, small, irregular kidneys that were painful on palpation. The cat was anemic with a packed cell volume of 19% and a total protein of 6.6 g/dL. Chronic kidney disease was suspected as the primary differential. Due to the poor prognosis, the patient was euthanized and submitted for a postmortem examination.

Gross evaluation confirmed small, firm kidneys with multifocal flat depressions in the capsular surface. Focally within the apex of the urinary bladder was a tan, well demarcated, raised, irregular plaque measuring 1.0cm x 1.5cm. Histopathology of the urinary bladder identified marked expansion of the lamina propria by diffuse infiltrates of histocytes with abundant granular eosinophilic cytoplasm consistent with von Hansemann-type macrophages which contained numerous intracytoplasmic basophilic inclusions of various sizes (Michaelis-Gutman bodies). The Michaelis-Gutmann bodies were positive for periodic acid-Schiff. This finding is consistent with the diagnosis of malakoplakia.

Malakoplakia is a rare disease in veterinary species, first identified in pigs. Since 2008, there have been three documented reports in the urinary bladders of cats and 8 reported cases in dogs. The histologic appearance is similar to canine granulomatous colitis seen in Boxers and French Bulldogs suggesting a possible deficient immune response to bacterial infection. Malakoplakia should be considered as a differential in young animals that present for dysuria that do not respond to antibiotic treatment.

Sunday, November 17, 2024
04:34 PM – 04:42 PM

NON-CHYLOUS LYMPHORRHAGIC PLEURAL EFFUSION AND POLYCYSTIC LIVER DISEASE IN A DOG

Bianca de Cecco[1,2], Jocelyn Lee[1], Andrea Johnston[1], Emi Sasaki[1,2]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]LSU-LADDL, Baton Rouge, LA, USA

An 8-year-old female spayed Maltese dog presented for inappetence, non-productive cough, and increased respiratory rate. Diagnostic imaging identified bilateral pleural effusion, an enlarged liver with multiple cystic structures, and a cystic soft tissue structure adjacent to the pancreas. Analysis of the pleural fluid was consistent with non-chylous lymphorrhage with acute hemorrhage. Despite placement of a pleural-port and octreotide therapy, the owner elected humane euthanasia after just 10 days of therapy. A postmortem examination was performed. On gross examination, the thoracic cavity contained approximately 250 ml of opaque fluid and atelectatic lungs. Hepatomegaly with marked distention of the hepatic parenchyma by numerous cysts was also observed. The kidneys and pancreas were normal. Histologically, liver cysts were lined with flat to plumped cuboidal CK7 positive epithelium, consistent with biliary epithelium. No significant lesions were observed in the pleura or diaphragm. This case was diagnosed as polycystic liver disease and subacute pleural effusion. Polycystic liver disease without associated polycystic kidney disease is rare in dogs and uncommon in humans. It is characterized by isolated cysts disrupting the hepatic parenchyma. Mechanistically, pleural lymphorrhagic effusion develops secondary to altered permeability of local capillaries and lymphatic vessels on the diaphragmatic pleural surface. Exudative pleural effusions are rare complications of polycystic liver disease. Only 3 human cases have been reported, all of which responded to surgical cyst decompression.

Sunday, November 17, 2024
04:42 PM – 04:50 PM

MALIGNANT SEMINOMA WITH SYSTEMIC METASTASES IN A RED RIVER HOG (POTAMOCHOERUS PORCUS)

Hernando Acevedo[1], Michael Garner[2], Jordan Davis-Powell[3], Stephanie French[4], Dalen Agnew[4], Anibal Armien[5], Javier Asin[1], Francisco Uzal[1], Eileen Henderson[1]
[1]California Animal Health and Food Safety (CAHFS) Laboratory System, San Bernardino Branch, University of California-Davis, San Bernardino, CA, USA, [2]Northwest ZooPath, Monroe, WA, USA, [3]Los Angeles Zoo, Los Angeles, CA, USA, [4]Michigan State University Veterinary Diagnostic Laboratory, East Lansing, MI, USA, [5]California Animal Health and Food Safety (CAHFS) Laboratory System, Davis Branch, University of California-Davis, Davis, CA, USA

A16-year-old red river hog (*Potamochoerus porcus*) was submitted to the San Bernardino branch of the California Animal Health and Food Safety Laboratory system for necropsy with a history of weight loss and ascites. This animal had undergone bilateral orchiectomy two months prior due to enlarged testicles, and had been diagnosed with bilateral seminoma with intravascular invasion. On postmortem examination, there were numerous soft, tan white raised masses measuring ~3-20 cm in greatest dimension throughout the serosa of the small intestines and stomach, capsular surface of the spleen and liver, diaphragm, and the parietal pleura (including the surface of multiple ribs). The largest mass was noted in the right inguinal canal with infiltration of the surrounding skeletal muscle, and with compression and displacement of the surrounding organs, including the urinary bladder. Histologic, transmission electron microscopy, and cytologic findings were consistent with a seminoma. Immohistochemistry was performed on samples of the primary testicular masses and the metastases. Samples of the masses obtained during necropsy and from the orchiectomy were negative for OCT3/4. Seminomas are germ cell tumors that are thought to arise from transformed gonocytes (prespermatogonia and spermatogonia), and usually occur in older animals. Reports of testicular tumors in suids are uncommon and, to the best of our knowledge, this is the first report of metastatic seminoma in a red river hog.

Sunday, November 17, 2024
04:50 PM – 04:58 PM

HEMANGIOSARCOMA AND PLASMA CELL NEOPLASIA IN THE RIGHT AURICLE OF A DOG

Patrice Witschen, Kelly Hughes
Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Background: A 10-year-old castrated male Golden Retriever enrolled in the Morris Animal Foundation Golden Retriever Lifetime Study presented to Colorado State University (CSU) Veterinary Diagnostic Laboratory for necropsy following acute collapse. The patient declined over 48 hours despite two blood transfusions and was humanely euthanized. The dog was a long-term patient of CSU, having a 10-month history of cardiac arrhythmias with a right auricular mass (managed with sotolol, radiation, and chemotherapy) and a 6-8 month history of hepatic and cutaneous plasma cell neoplasia diagnosed with cytology and the same immunoglobulin clonal rearrangements at both sites via PARR. The patient maintained normal globulins throughout disease.

Results: Post-mortem exam revealed 2L of frank blood in the abdominal cavity. There were multifocal variably soft to fluctuant cavitated dark red raised nodules scattered throughout the heart (right auricle), lung, liver, kidney, jejunum, omentum, spleen, adrenal gland, cerebrum, and cerebellum. Histologic evaluation of the right auricle revealed sheets of neoplastic round cells trickling through irregular, arborizing vascular structures lined by neoplastic spindle cells (endothelium). Due to the dichotomy of the cellular population, additional diagnostics were performed; round cells showed

nuclear immunoreactivity for MUM1 while arborizing vascular structures were negative for this antigen. Neoplastic plasma cells were also confirmed in the liver.

Conclusion: The current case is a unique report describing intermingling of a hemangiosarcoma and plasma cell neoplasia in the right auricle of a dog. This is also an unusual presentation of disseminated plasma neoplasia lacking other evidence of systemic disease.

Monday, November 18, 2024
08:00 AM – 08:07 AM

ARTIFICIAL INTELLIGENCE IS ABLE TO PREDICT C-KIT MUTATIONS IN HE SLIDES OF CANINE MAST CELL TUMORS – ARE WE?

Robert Klopfleisch
Freie Universität Berlin, Berlin, Germany

Background

The internal tandem duplication mutation in c-Kit exon 11 (ITD) is a clinically significant mutation in veterinary oncology. This project aimed to train a deep learning algorithm (DLA) to identify ITD status using only HE slides. Additionally, it explored whether the DLA's capabilities could be transferred to pathologists through reverse computing.

Materials and Methods

HE slides from 196 c-Kit-11 mutated and 189 non-mutated mast cell tumors (MCTs) were used to train a DLA based on multiple instance learning from whole slide images. The DLA was then tested to predict ITD status from HE slides. In a subsequent ongoing study, pathologists were tasked with predicting ITD status from MCT images. They were then trained using images of tumor areas identified by the DLA as highly relevant for ITD prediction. Finally, the trained pathologists were asked to predict ITD status for a new set of MCT images.

Results

The DLA accurately predicted ITD status in HE-stained MCTs in 83% of cases. Five untrained pathologists correctly classified ITD status in 43–88% of images. However, after training, their performance decreased to 25-56% correct classifications in a new set of images.

Conclusions

The DLA-assisted morphological examination of MCTs can quickly and accurately predict c-Kit-11 mutational status, potentially reducing the time associated with PCR analysis. However, the current results suggest that transferring the algorithm's skills to human observers is challenging. A specific morphological feature of ITD-positivity derived from the AI model that is usable by human observers remains to be identified.

Monday, November 18, 2024
08:07 AM – 08:14 AM

HAIR AS AN ALTERNATIVE SOURCE OF HIGH YIELD CANINE GENOMIC DNA

Mackenzie Long, Tori Rudolph, Leigh Anne Clark, Tina Meichner
University of Georgia College of Veterinary Medicine, Athens, GA, USA

Background: To ensure adequate assessment of somatic mutations within tumor tissue, an appropriate germline reference tissue must be selected. Blood is the preferred source of genomic DNA. However, presence of circulating neoplastic cells could obscure identification of somatic

variants. While buccal swabs also serve as a source of genomic DNA, they may have similar neoplastic cell content. For this reason, germline testing in humans may employ DNA derived from cultured skin fibroblasts, hair bulbs or bone marrow-derived mesenchymal stromal cells. **Objective:** To evaluate whether DNA derived from canine hair bulb samples provides sufficient depth and coverage for whole genome sequencing (WGS) and resolution of heterozygous variants. **Methods:** Plucked hair samples were collected from dogs of various breeds. Hair was cut closest to the follicle end and approximately 10 mg submerged in lysis buffer containing dithiothreitol and proteinase K. Following overnight incubation (56ºC with agitation), DNA was purified from the supernatant using a modified protocol of the Qiagen QIAamp DNA blood kit. DNA quality and quantity was assessed with a spectrophotometer. WGS was performed on three samples using a standard 350bp DNA library (Illumina). Sequences were aligned to canfam4 and genotypes were determined for the highly polymorphic locus, Dog Leukocyte Antigen (DLA)-DRB1 using Integrated Genome Viewer. **Results/Conclusion:** DNA derived from canine hair bulbs resulted in average fold coverage ranging from 30X-39X, which was sufficient for resolving DLA-DRB1 genotyping. Future studies will evaluate whether DNA derived from canine lymphoma patient hair bulbs provides enhanced somatic variant detection over that of peripheral blood.

Monday, November 18, 2024
08:14 AM – 08:21 AM

ORAL FIBROLIPOMA IN DOGS: RETROSPECTIVE CASE SERIES STUDY AND COMPARATIVE REVIEW

Daniel Felipe Barrantes Murillo[1], Alexis Berrocal[2], Cynthia Bell[3], Daniel R. Rissi[4], Linden E. Craig[5], Erin A. Graham[6], Emily J. Brinker[7], Tatiane Terumi Negrão Watanabe[8]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]Laboratorio de Patología Veterinaria Histopatovet, Heredia, Costa Rica, [3]Specialty Oral Pathology for Animals, LLC, Geneseo, IL, USA, [4]Athens Veterinary Diagnostic Laboratory, Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [5]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA, [6]Tifton Veterinary Diagnostic and Investigational Laboratory, College of Veterinary Medicine, University of Georgia, Tifton, GA, USA, [7]Department of Comparative Pathobiology, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA, [8]Antech Diagnostics, Mars Petcare Science & Diagnostics, Fountain Valley, CA, USA

Background: Fibrolipoma is defined as a typical lipoma transected by variable amounts of paucicellular and collagenous fibrous components. Oral and lingual fibrolipomas are well-recognized histological entities in human medicine, slightly more prevalent in females, after the fourth decade and arising from the buccal mucosa. The documentation of this neoplasm in the oral cavity is lacking in veterinary medicine.

Objective: To document the clinical and pathological features of oral fibrolipoma in dogs.

Methods: Through a multi-institutional retrospective compilation of cases submitted to diagnostic pathology services, here we describe the clinical and pathologic features of oral fibrolipomas in dogs.

Results: A total of 112 cases of oral fibrolipomas in dogs and 3 cases in cats were retrieved. In dogs, the mean age was 10.1 years (range 2-16 years, ±2.63 years standard deviation, with an average tumor size of 1.7 cm (range 0.2- 8 cm, ±1.1 cm standard deviation). The most common location was the tongue (57.1%, 64/112, followed by the buccal mucosa (15.2%, 16/112), sublingual area (8.0%, 9/112), gingiva and lip (4.5%, 5/112 each), and palate (1 case). The anatomical location of the oral fibrolipoma only differ significantly among the dog breeds ($P<0.001$) but not among sex, age, anamnesis, or reason for submission. The tumor was most reported in males (69.7%, 78/112) and in 62.5% (70/112) of the cases, the tumor was an incidental finding.

Conclusions: Fibrolipoma should be considered a differential diagnosis when considering benign lingual and other oral soft tissue masses in dogs.

Monday, November 18, 2024
08:21 AM – 08:28 AM

UNILATERAL PECTORAL MUSCLE INFARCTION: A UNIQUE MANIFESTATION OF FUNGAL INFECTION IN BIRDS

Shotaro Nakagun, Garrett Fraess, Rachel Burns
San Diego Zoo Wildlife Alliance, San Diego, CA, USA

Fungal infection, especially respiratory aspergillosis, is a major cause of mortality in birds. This study describes the clinicopathologic features of a unique manifestation of fungal infection, in which arteritis and thrombosis at the brachiocephalic trunk causes unilateral pectoral muscle infarction. A retrospective search in the San Diego Zoo Wildlife Alliance database identified 47 cases between 1988–2023. The affected birds were passerines (n = 44), psittacines (n = 2), and a piciform (n = 1). There were 33 affected species across 18 families, including 45 collection birds and 2 wild birds. Birds of the family Sturnidae (starlings and mynas) were overrepresented (n = 16). Fifteen birds were presented deceased and 32 birds were presented for veterinary care, most often with a unilateral wing droop (n = 25). The left pectoral muscle was more frequently infarcted (n = 31). Pronounced asymmetric pectoral muscle pallor was pathognomonic for this condition grossly even if arteritis or other fungal lesions were not initially evident. Histologically, fungal invasion of blood vessel walls was associated with necrosis, inflammation, and thrombosis. *Aspergillus* spp. were identified histologically in 46 cases and confirmed by PCR and/or culture in 7 cases, whereas 1 case was histologically consistent with mucormycosis. These lesions were considered extensions from a primary clavicular air sac infection, and involvement of lung or other tissues was limited (n = 18). No overt trends were found with sex, reproductive status, comorbidities, seasonality, year, relocation history, or habitat. The study raises awareness of this unusual disease presentation.

Monday, November 18, 2024
08:28 AM – 08:35 AM

HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N1 CLADE 2,34B IN A FREE-RANGING ALASKAN POLAR BEAR (URSUS MARITIMUS)

Raphaela Stimmelmayr[1,2], David Rotstein[3], Mia Torchetti[4], Robert Gerlach[5]
[1]North Slope Borough Department of Wildlife Management, Barrow, AK, USA, [2]University of Alaska Fairbanks, Fairbanks, AK, USA, [3]Marine Mammal Pathology Services, Olney, MD, USA, [4]US Department of Agriculture Animal and Plant Health Inspection Service National Veterinary Service Laboratories, Ames, IA, USA, [5]Alaska Department of Environmental Conservation, Anchorage, AK, USA

Background: Highly pathogenic avian influenza virus, H5N1, clade 2.3.4.4b of the H5 subtype emerged in 2020 and has spilled over from avian species to mammals. There have been sporadic reports in free ranging bears within North America including American black bears (*Ursus americanus*), grizzly bears (*U. arctos horribilis*), and Kodiak bears (*U. arctos middendorffi*). We report a case of naturally occurring H5N1 in a free-ranging polar bear (*U. maritimus*) in Alaska.

Objective: Detail the gross, histopathologic, immunohistochemical findings of HPAI infection in a polar bear.

Methods: Gross necropsy and histopathologic evaluation were conducted. Swabs (oral, nasal, rectal, brain) were collected for Influenza A viral PCR. Formalin fixed-paraffin embedded sections of brain were submitted for confirmatory PCR testing and viral genome characterization at the National Veterinary Service Laboratory.

Results: The animal was a young male polar bear, found dead and in poor body condition. There were unilateral periocular ulcerations, hepatic and pulmonary congestion, empty stomach, and cerebral swelling and congestion. The primary histopathologic finding was a granulocytic and mononuclear meningoencephalitis with microgliosis, neuronal necrosis, vasculitis, and parenchymal rarefaction. Swabs were negative for Influenza A. Influenza A immunohistochemistry detected antigen in neuronal cytoplasm. HPAI H5N1 Genotype A3 (unreassorted fully Eurasian lineage), clade 2.3.4.4b was identified.

Conclusion: The source of exposure was not determined, but HPAI-positive short-tailed shearwaters (*Puffinis tenuirostris*) were found in the North Slope, AK; bird predation and eggs have been observed in polar bears. No additional cases have been observed and monitoring continues.

Monday, November 18, 2024
08:35 AM – 08:42 AM

HEMORRHAGIC SEPTICEMIA IN MINNESOTA BOVINE CALVES

Heidi Pecoraro, Brett Webb, Brianna Stenger, Kelli Maddock
North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND, USA

Background: *Pasteurella multocida* capsular type B, the causative agent of hemorrhagic septicemia (HS), has rarely been reported in North American livestock since the last outbreak of disease in 1993 in California beef calves.

Objective: To describe the gross and histopathologic lesions in two naturally occurring cases of bovine HS associated with *P. multocida* capsular type B infection.

Methods: Postmortem examinations were performed on one 5-month-old, Black Angus heifer calf and one 2.5-month-old, Bison (*Bison bison*) bull calf at the North Dakota State University Veterinary Diagnostic Laboratory. Ancillary testing, including bacterial cultures and molecular analyses, was also completed.

Results: On gross examination, the beef calf had diffuse pulmonary edema and congestion, along with fibrinous pericarditis. There were scattered petechia/ecchymoses within or along the subcutis, thoracic serosal surfaces, and skeletal musculature. Edema, hemorrhage, and fibrin were present within abdominal tissues and the body wall of the bison calf. Pulmonary hemorrhages and arthritis were also observed. Microscopically, for both calves, tissues were variably expanded by hemorrhage, edema, and fibrin. Respiratory cultures isolated *P. multocida* for both calves, while enteric cultures from the bison calf were positive for the bacterium as well. Capsular type B by *bcbD* PCR was positive for all isolates. Further sequencing analysis identified the isolates as LPS genotype L3, consistent with Heddleston serovars 3,4.

Conclusion: Because HS is a possible re-emerging disease, bovine cases positive for *P. multocida* with fibrinosuppurative serositis and widespread hemorrhage but no significant bronchopneumonia should be suspicious for HS.

Monday, November 18, 2024
08:42 AM – 08:49 AM

CARDIAC LESIONS ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS TYPE D ENTEROTOXEMIA IN SHEEP AND GOATS

Emma Torii[1], Omar Gonzales-Viera[1], Jennine Ochoa[2], Todd Cornish[2], Francisco Uzal[3], Asli Mete[1]
[1]California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California - Davis, Davis branch, Davis, CA, USA, [2]California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California - Davis, Tulare branch, Tulare, CA,

USA, [3]California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California - Davis, San Bernardino branch, San Bernardino, CA, USA

Background: Lesions associated with enterotoxemia caused by *Clostridium perfringens* type D have been well described in sheep, and to a lesser extent in goats. There is limited information available on cardiac lesions associated with spontaneous cases of enterotoxemia in both species. **Methods and Results:** A retrospective review of sheep and goats submitted to the California Animal Health and Food Safety Laboratory System (January 2013- May 2024) identified 98 animals that were positive for epsilon toxin in the small and/or large intestinal contents by ELISA, and where heart was histologically examined. Of these, 17 cases (17%; 15 necropsy and 2 tissue-only submissions) had histological cardiac lesions that were presumed to be secondary to enterotoxemia. Ten sheep and seven goats were identified with such lesions. Ten were juvenile (<one year old), whereas seven were adults. Cardiac lesions included hydropericardium (7/15, 47%) on gross examination; and myocardial degeneration and/or necrosis (13/17, 76%), proteinaceous myocardial interstitial edema (6/17, 35%), and hemorrhage (14/17, 83%) on histology. Hydropericardium and proteinaceous myocardial interstitial edema were only observed in sheep. Myocardial necrosis and/or degeneration was present in both species, but with a higher prevalence in goats compared to sheep (7/7, 100% vs 6/10, 60%, respectively). Hemorrhage was more frequent in sheep compared to goats (10/10, 100% vs 4/7, 57%, respectively). **Conclusions:** Cardiac lesions occur in many spontaneous cases of *C. perfringens* type D enterotoxemia in small ruminants, and should be considered a differential when hydropericardium, cardiac necrosis/degeneration, interstitial proteinaceous edema, and/or hemorrhage are observed.

Monday, November 18, 2024
08:49 AM – 08:56 AM

MITOCHONDRIAL MYOPATHY IN A LITTER OF GERMAN SHEPHERD DOGS

Hayley Hunt[1], Matthew Perrott[1], Andrew Worth[1], Anita Shea[1], Nick Cave[2], Vicki Erceg[1]
[1]School of Veterinary Science, Massey University, Palmerston North, New Zealand, [2]VetSciNC, Palmerston North, New Zealand

Mitochondrial diseases are a heterogenous group of disorders affecting organs that are heavily dependent on oxidative metabolism, and those that primarily affect skeletal muscle are termed mitochondrial myopathies. In humans, mitochondrial myopathies are well-described and are reported to affect 5-15/10000 people, but only a small number of cases are reported in dogs. Four out of seven German Shepherd puppies in a litter from a working dog line in New Zealand developed exercise intolerance and panting with exercise starting from 5.5 months of age. The clinical signs seen were variable, ranging from occasional excessive panting through to slowing or stopping during exercise, sometimes with vocalization in pain.

All four affected dogs had moderate to severe increases in creatine kinase (range 3445 – 190150 IU/L), aspartate aminotransferase (259 – 3603 IU/L) and alanine aminotransferase (282 – 837 IU/L) activities in serum samples taken immediately after 5 minutes of strenuous exercise, indicating muscle damage. Enzyme activities remained near to the normal range in the unaffected siblings. Histologically, biopsies from the biceps femoris, vastus lateralis, forelimb and epaxial muscles of the worst affected dog showed occasional myocytes undergoing degeneration, regeneration, or fibre splitting. Electron microscopy showed an increased number of mitochondria in the sub-sarcolemmal space adjacent to the nucleus, along with large and abnormally shaped mitochondria. These findings are consistent with a mitochondrial myopathy. At 18 months of age, the affected puppies have shown minimal progression of symptoms and minimal muscle atrophy.

Monday, November 18, 2024
08:56 AM – 09:03 AM

GASTROINTESTINAL BIOPSIES IN HORSES: HISTOLOGICAL AND IMMUNOLOGICAL COMPARISON WITH FULL-THICKNESS TISSUE SAMPLING

Daniel Jean[1], Camille Ruault[1], Juliette Monbrun[1], Tristan Juette[1], Marie-Odile Benoit-Biancamano[1], Nanny Wenzlow[2]
[1]University of Montreal, Faculty of Veterinary Medicine, St-Hyacinthe, QC, Canada, [2]Texas Tech University, School of Veterinary Medicine, Amarillo, TX, USA

Background: The histologic interpretation of gastrointestinal biopsies remains a challenge in horses, as standards are lacking for these procedures.

Objectives: The study aimed to describe and compare the histological and immunological findings in biopsy and full-thickness gastrointestinal (GI) segments in horses.

Methods: Nine horses (6 females, 3 geldings; 5 to 28 years old) donated to the University of Montreal, without gastrointestinal conditions, were used. Full-thickness and mucosal biopsies obtained using endoscopic forceps were taken postmortem from the duodenum, jejunum and rectum in each horse. Standard hematoxylin eosin phloxine saffron (HEPS) sections from all GI tissues underwent a blind histologic evaluation by two board-certified veterinary pathologists. Immunohistochemically stained slides allowed histomorphometrical counts (absolute numbers per field) of B (CD20) and T lymphocytes (CD3) within the duodenal and rectal epithelium, the lamina propria (apical and basal areas) and the subcryptal area (rectum only); biopsy samples were compared with three sections of their corresponding full-thickness duodenal and rectal counterparts of equivalent size. Results were analyzed using intra-class correlation.

Results: Preliminary data show that biopsies tend to under-estimate the number of lymphocytes and plasma cells in the duodenal lamina propria compared to their full-thickness counterparts. Eosinophils are more prevalent in the jejunum compared to the duodenum. Rectal biopsies tend to have lower counts of neutrophils and macrophages compared to their full-thickness counterparts.

Conclusions: These variations in immune cell distribution in the different sections of the horses' GI tract must be taken into consideration during histopathological evaluation.

Monday, November 18, 2024
09:03 AM – 09:10 AM

IMPLEMENTATION OF TISSUE MICROARRAYS IN A PRIVATE DIAGNOSTIC LABORATORY TO IMPROVE OPTIMIZATION AND VALIDATION OF DIAGNOSTIC IHC PROTOCOLS AND RETROSPECTIVE STUDIES

Jennifer Ward, Karla Pedroza
SpecialtyVETPATH, Seattle, WA, USA

Background: Tissue microarrays (TMAs) are paraffin blocks in which tissue cores are assembled, allowing assays to be performed on multiple samples simultaneously. TMAs have become an integral part of diagnostic and research immunohistochemistry (IHC) in human medicine; yet, while they are increasingly implemented in veterinary medicine, TMAs are still underutilized, and limited in availability.

Objective: We describe the development of a TMA program implemented in our private diagnostic laboratory, with a goal of introducing these methods and the associated benefits to other laboratories.

Methods: TMA blocks were created by manual core transfer using pre-poured TMA recipient blocks and cores of formalin-fixed, paraffin-embedded (FFPE) tissue. Donor blocks were obtained from SpecialtyVETPATH's archive. H&E-stained slides of potential donor blocks were screened by board-certified veterinary pathologists and the area of interest was delineated on the slide. Following construction, slides were prepared and subjected to immunostaining, and results were analyzed for validation of antibodies in diagnostic assays. Validated antibodies were subsequently used in routine diagnostic IHC. TMA slides were also utilized for research projects, allowing investigators to perform assays on tissues from multiple patients simultaneously on one slide.

Conclusions: The use of TMAs in development of diagnostic assays enhances reliability and reduces investment cost for both assay development and retrospective investigations. An added value is to allow mining of tissue archives for data, which, when correlated with outcomes, can contribute to understanding of tumor behavior and identification of predictive biomarkers. Thus, use of TMAs supports good stewardship and good laboratory practices.

Monday, November 18, 2024
09:10 AM – 09:17 AM

PATHOLOGY OF LAGOVIRUS EUROPAEUS GI.2 (RHDV2) AND GII.1 (EBHSV) INFECTIONS IN WILD EUROPEAN BROWN HARES, LEPUS EUROPAEUS, IN ENGLAND

Katherine Hughes[1], Jonathan Day[1], Frank Jiggins[1], Diana Bell[2]
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of East Anglia, Norwich, United Kingdom

Background: *Lagovirus europaeus* GI.2 (rabbit haemorrhagic disease virus 2; RHDV2) and GII.2 (European brown hare syndrome virus; EBHSV) both infect wild European brown hares, *Lepus europaeus*. However, detailed contemporary descriptions of the pathology associated with these viruses in hares in England are sparse.
Objective: To describe a case series of European brown hares diagnosed with RHDV2 or EBHSV by PCR.
Methods: Hares were identified through a project investigating causes of morbidity and mortality in UK hares reported dead by citizen scientists. Hares underwent a post mortem examination, and a diagnosis of *Lagovirus europaeus* GI.2 or GII.2 was made following a positive PCR result on fresh liver.
Results: Five hares reported dead between November 2018 and April 2024 were positive for EBHSV, and one hare was positive for RHDV2. Four hares were female, one was male, and the sex was not recorded for one animal. The female diagnosed with RHDV2 was lactating. Mean body weight was 2.66 kg (median: 2.49 kg). All animals were from counties in the east of England, likely representing a study bias. Amongst the most consistent findings were macroscopic subtle, multifocal, hepatic pallor; multifocal to coalescing hepatic necrosis; thymic lymphocytolysis and profound tracheal congestion. Cases of EBHSV frequently exhibited hepatic infiltrates of small numbers of lymphocytes and plasma cells, and rare heterophils, and multifocal, mild mineralization of hepatocytes.
Conclusions: EBHSV and RHDV2 have considerable overlap in pathological features but we present evidence to support the assertion that hepatic necrosis is more chronic in EBHSV.

Monday, November 18, 2024
09:17 AM – 09:24 AM

GASTRIC PLASMA CELL TUMORS IN DOGS

Paula Giaretta[1], João Cavasin[1], Madison Mascorro[1], Simon Priestnall[2], Shelley Newman[3]
[1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA, [2]Department of

Pathobiology & Population Sciences, The Royal Veterinary College, Hatfield, United Kingdom, [3]Newman Specialty VetPath, Hicksville, NY, USA

Background: Extramedullary plasma cell tumors are usually benign tumors found in the skin or oral cavity of dogs. In the digestive system, they typically occur in the colon or rectum, although isolated cases have been reported in the stomach.

Objective: To describe the pathologic and clinical presentation of gastric plasma cell tumors in dogs.

Methods: The records from 2011 to 2023 of the Histopathology Service at the Gastrointestinal Laboratory of Texas A&M University were searched for cases of gastric plasma cell tumors in dogs. Immunohistochemistry assays and PCR for immunoglobulin heavy chain rearrangements (PARR) were performed.

Results: Twelve cases of gastric plasma cell tumors were identified, all located in the antrum. Grossly, findings included a single mass-like lesion in 6/12 cases, multiple masses in 4/12 cases, and diffuse mucosal thickening in 2/12 dogs. Nine dogs presented with chronic vomiting or regurgitation, and one dog had anemia. In two dogs, the plasma cell tumor was incidental. Seven dogs had moderate to marked chronic gastritis. Immunohistochemically, neoplastic cells were positive for MUM-1, variably positive for CD20, and negative for PAX5 and CD3. PARR was performed in seven cases, revealing three with a clonal population within a polyclonal background, three polyclonal cases, and one non-diagnostic result.

Conclusions: Gastric plasma cell tumors in dogs typically occur in the antral mucosa. Chronic gastritis is a frequent concomitant lesion, suggesting that gastric plasma cell tumor may arise in areas of chronic immune cell stimulation.

Monday, November 18, 2024
09:24 AM – 09:31 AM

MICHAELIS-GUTMANN BODIES IN ESCHERICHIA COLI-ASSOCIATED GRANULOMATOUS COLITIS IN DOGS

Paula Giaretta[1], João Cavasin[1], Madison Mascorro[1], Raquel Rech[2]
[1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, School of Veterinary Medicine and Biomedical Sciences, College Station, TX, USA, [2]Department of Veterinary Pathobiology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Background: Michaelis-Gutmann bodies are mineralized inclusions characteristic of malakoplakia, a rare granulomatous disease that affects immunosuppressed humans. In dogs, malakoplakia has been described in the urinary bladder, with only one reported case in the large intestine. Histologic lesions of malakoplakia resemble those of *E.coli*-associated granulomatous colitis, however, Michaelis-Gutmann bodies have not been investigated in the latter.

Objective: To investigate the presence of Michaelis-Gutmann bodies in cases of *E. coli*-associated granulomatous colitis in dogs.

Methods: The records of the Histopathology Service at the Gastrointestinal Laboratory of Texas A&M University were searched for cases of *E. coli*-associated granulomatous colitis/histiocytic ulcerative colitis confirmed by fluorescence in situ hybridization. Periodic-acid Schiff (PAS), Perl's Prussian blue, and von-Kossa stains were performed.

Results: Fifty-three cases of *E. coli*-associated granulomatous colitis were identified. A variable number of Michaelis-Gutmann bodies were identified in colonic lesions of 15 out of 53 cases (28%). The inclusions were in the cytoplasm of macrophages or extracellular, spheroid to targetoid, ranging from 2-11 µm diameter with an average size of 4 µm. Michaelis-Gutmann bodies varied from basophilic to eosinophilic on hematoxylin and eosin stain and were positive on PAS, Perl's Prussian blue, and von-Kossa stains.

Conclusions: Michaelis-Gutmann bodies are occasionally present in lesions of *E. coli*-associated granulomatous colitis in dogs. Because these bodies are PAS positive, they can potentially be confused with yeasts or algae. Stains for iron and calcium can be used to differentiate between Michaelis-Gutmann bodies and infectious organisms.

Monday, November 18, 2024
09:31 AM – 09:38 AM

LESIONS OF MYCOBACTERIUM AVIUM SPP. HOMINISSUIS INFECTION MIMICS CORYNEBACTERIUM PSEUDOTUBERCULOSIS INFECTION LESIONS IN GOATS

Ida Phillips, HEATHER WYSS
Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC, USA

A four-year-old intact female LaMancha goat presented to the Rollins Animal Disease Diagnostic Laboratory for necropsy after an approximately 4 month history of weight loss and masses from the poll and udder. She presented to the North Carolina College of Veterinary Medicine after having increased respiration rate and effort and was euthanized after not improving. Gross examination revealed multiple abscesses from the poll, the caudodorsal lung lobes, tracheobronchial lymph nodes, mesenteric lymph nodes, inguinal lymph nodes, and mammary glands. Histopathology found severe, chronic, necrosuppurative and caseating lymphadenitis, pneumonia, mastitis, and dermatitis with multifocal areas of mineralization. Necrosis and granulomatous inflammation was found in the heart, spinal cord, small intestine, liver, kidney and spleen. Special stains for fungi and Mycobacterial organisms were performed. No fungal organisms were identified using a PAS stain while the Fites and Ziehl Neelsen acid-fast stains revealed an abundance of rod-shaped, acid-fast organisms within macrophages and free among the necrotic cellular debris. Sequencing & Identification on Formalin-Fixed Paraffin Embedded Tissue were performed at the University of Georgia Athens Veterinary Diagnostic Laboratory. Sequencing identified *Mycobacterium avium subsp. Hominissuis.* The lesions in this case were morphologically similar to those described in animals infected with *Corynebacterium pseudotuberculosis,* the causative agent of Caseous Lymphadenitis (CLA). Bacteriological results were negative for *Corynebacterium pseudotuberculosis.* Although the presence of abscesses in the peripheral lymph nodes of small ruminants is highly suggestive of CLA, this case illustrates the importance of special stains, bacterial cultures, and molecular diagnostics in gaining a definitive diagnosis in suspected cases.

Monday, November 18, 2024
10:15 AM – 10:22 AM

TRANSFORMING VETERINARY DIAGNOSTICS: HOW ARTIFICIAL INTELLIGENCE ENHANCES CLINICAL DECISION-MAKING

Arun Apte[1], Mrinal Kanti Chatterjee[2], Garima Batra[2]
[1]CloudLIMS.com, Wilmington, DE, USA, [2]CloudLIMS Lab Solutions (India) Pvt. Ltd., Indore, India

Problem Statement: Recent advancements in diagnostic tools and the increasing complexity of the data they generate are complicating clinical decision-making, and delaying zoonotic disease diagnosis, monitoring, and treatment. For example, modern vet hematology analyzers can analyze hundreds of parameters in each blood sample and generate results. In imaging modalities, such as MRI, CT, PET, and ultrasound, the inability to consistently detect minute abnormalities in medical

images can lead to missed diagnoses and delayed treatments. Additionally, traditional pathogen detection and epidemiological surveillance methods are often too slow to identify emerging disease patterns, delaying timely interventions and allowing outbreaks to spread.

Proposed Solution: Artificial Intelligence (AI) can analyze numerous parameters in minimal blood samples, providing comprehensive information and supporting clinical decision-making. In imaging, AI can enhance the interpretation of X-rays, CT scans, and ultrasounds, detecting subtle abnormalities and improving treatment outcomes. AI-driven tools reduce analysis time and noise, enabling quicker decision-making. Moreover, AI advances disease surveillance by analyzing diverse datasets to identify and predict outbreaks, facilitating timely interventions. A Laboratory Information Management System (LIMS) supports AI-driven diagnostics through robust data management, high-quality data driven by automation, and advanced analytics and reporting, ensuring reliable outcomes.

Conclusion: Integrating AI into veterinary diagnostics and disease surveillance addresses critical inefficiencies and enhances diagnostic accuracy. This transformation enables veterinarians to make quicker, more informed decisions, ultimately improving animal health outcomes and disease management. Leveraging AI and LIMS, veterinary diagnostics can achieve a new standard of precision, efficiency, and proactive care.

Monday, November 18, 2024
10:22 AM – 10:29 AM

GRANULOMATOUS HEPATITIS IN A CHACOAN HORNED FROG (CERATOPHRYS CRANWELLI) WITH AMPHIBIAN-TYPE BRUCELLA

Aimee Pepper[1], Shih-Hsuan Hsiao[1], Judilee Marrow[1], Aubrey Engel[1], Martha Delaney[2], Gretchen Anchor[2], Chien-Che Hung[1]
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Zoological Pathology Program, Brookfield, IL, USA

Background: *Brucella* range in pathogenicity in amphibians, with animals displaying subclinical infection, localized skin lesions, or even fulminant systemic disease. The zoonotic potential of amphibian-type *Brucella* is largely unknown, though recent isolation of a *Brucella inopinata*-like strain from an animal keeper with brucellosis suggests the possibility of interspecies transmission. To our knowledge, *Brucella* has only been isolated from one other Chacoan horned frog with bilateral panophthalmitis.

Objective: To characterize a case of granulomatous hepatitis in association with amphibian-type *Brucella* in a Chacoan horned frog submitted for necropsy.

Results: Grossly, the subcutaneous tissues and coelomic cavity exuded abundant yellow, clear, watery fluid (subcutaneous edema and ascites). The liver contained innumerable white, firm, miliary nodules. Histopathology revealed the presence of myriad randomly distributed granulomas with a core of necrotic debris that was further surrounded by epithelioid macrophages, lymphocytes and plasma cells, and rare multinucleated giant cells. Aerobic culture of the liver yielded growth of *Brucella*, with confirmation by RT-PCR (Ct = 22.8). Speciation utilizing Bruce-ladder and Suis-ladder PCR identified atypical *Brucella*. Whole genome sequencing revealed that the isolate clustered in a clonal group with *Brucella* strains identified previously from other amphibians in the United States. GMS and Ziehl-Neelsen staining did not identify concurrent fungal organisms or *Mycobacterium*, and *Mycobacterium* PCR was negative. ISH revealed *Brucella* nucleic acid in association with hepatic granulomas.

Conclusions: To our knowledge, this is the first report of granulomatous hepatitis in association with *Brucella* in a Chacoan horned frog.

Monday, November 18, 2024
10:29 AM – 10:36 AM

INFANTILE CORTICAL HYPEROSTOSIS IN A RHESUS MACAQUE

Krystal Vail, Adam Myers
Tulane National Primate Research Center, Covington, LA, USA

A female, stillborn, Indian-origin rhesus macaque presented for necropsy. Grossly, the pelvic limbs were enlarged due to bilaterally symmetric, irregular, spongy, proliferative subperiosteal cortical expansion of the femur, tibia, and fibula diaphyses. The thoracic limbs and skull were unremarkable. Microscopically, the diaphyseal cortices were expanded by irregular trabeculae of immature woven bone interspersed with loose fibrous stroma and minimal inflammation. Subperiosteal new bone formation and incomplete epiphyseal ossification are features of infantile cortical hyperostosis, a condition that radiographically and histologically resembles Caffey's disease in humans. Infantile cortical hyperostosis-like disease has been reported in swine, a cat, and dogs, in addition to rare reports of familial and spontaneous disease in rhesus macaques. This report describes a case of spontaneous congenital cortical hyperostosis in a stillborn rhesus macaque.

Monday, November 18, 2024
10:36 AM – 10:43 AM

MICROCHIP ASSOCIATED CUTANEOUS MELANOPHOROMA IN AN ADULT WYOMING TOAD (ANAXYRUS BAXTERI)

Michelle Degnin, Allison Watson
Colorado State University, Department of Microbiology, Immunology, and Pathology, Fort Collins, Fort Collins, CO, USA

Introduction: Subcutaneous microchips have been used for identification of wildlife and domestic animals, for identification and in the study of life history, sex ratios, and migration. Subcutaneous microchips are less likely to be lost or damaged, and negative outcomes are usually minimal. Endangered Wyoming Toads (*Anaxyrus baxteri*) were kept with the Wyoming Ecological Services and injected with a microchip on the left flank. An approximately 5-year-old toad from this facility was submitted for necropsy after being found dead with a mass over the microchip site.

Methods: The whole toad was submitted in ethanol and a necropsy was performed. Histopathology was performed on the skin and body wall, lung, liver, kidney, and intestines. Grocott methenamine silver (GMS) was used on skin sections, and Acid-fast on visceral organs. The highly pigmented mass was bleached.

Results: An 18 x 17 x 10mm firm, elevated mass was identified over the microchip site. The mass was dark brown on cut section. Histopathologically, the mass was composed of streams of dark brown pigmented spindle to polygonal cells, consistent with melanophoroma. Additionally, cutaneous fungal hyphae were identified and confirmed with GMS.

Conclusions: Microchip associated neoplasia has been described in mammals, including domestic cats (*Felis catus*), dogs (*Canis familiaris*), and laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*). The close association of melanophoroma to the microchip suggests a possible relationship between the microchip and neoplasia. The fungal infection is consistent with opportunistic Zygomycetes. To our knowledge, this is the first report of a microchip associated neoplasm in amphibians.

Monday, November 18, 2024
10:43 AM – 10:50 AM

HEPATIC INFILTRATING LIPOMA IN A SHORT-EARED OWL

Carmen H. Lau[1], Arnaud J. Van Wettere[1], Sheri Iodice[1], Anibal G. Armien[2]
[1]Utah Veterinary Diagnostic Laboratory, Utah State University, Logan, UT, USA, [2]California Animal Health & Food Safety Laboratory System, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA

Infiltrating lipomas in any organ system are uncommon neoplasms in both human and veterinary medicine. An 8-year-old, male, short-eared owl in permanent residence at a rehabilitation facility since fledging stage was reported with a few days of weight loss and hyporexia before death. Necropsy demonstrated a firm, tan and brown mottled liver. Microscopic examination demonstrated coalescing sheets of mature adipocytes replacing approximately 70% of the hepatic parenchyma and extending between remaining hepatic cords. The hepatocytes immediately adjacent to the adipocytes contained moderate to large amounts of cytoplasmic vacuolation. Oil red O staining demonstrated lipid in the adipocytes and hepatocytes. Electron microscopy confirmed that the cells were adipocytes and not markedly lipid-laden hepatocytes. Infiltrating lipomas are rarely reported in livers in the human medical literature, and not reported in the veterinary literature. To the author's knowledge, this is the first reported case of a hepatic infiltrating lipoma in a veterinary species.

Monday, November 18, 2024
10:50 AM – 10:57 AM

HISTOLOGICAL AND IMMUNOLOGICAL STUDY OF GASTROINTESTINAL BIOPSIES IN HORSES WITH ACUTE AND CHRONIC COLIC

Patricia Arnaiz[1], Nanny Wenzlow[2], Guillaume St-jean[1], Daniel Jean[1]
[1]University of Montreal, Faculty of Veterinary Medicine, St-Hyacinthe, QC, Canada, [2]Texas Tech University, School of Veterinary Medicine, Amarillo, TX, USA

Background: Gastrointestinal infiltration is reported to be significantly increased in horses with recurrent colic.

Objectives: Describe the histological and immunological findings in horses with acute/chronic colic.

Methods: Included were colic horses with complete medical records (classical work-up for colic patients) that also had gastrointestinal biopsies taken at our Equine Veterinary Hospital (January 2022 - April 2023). Horses were categorized as chronic (2 or more episodes within < 6 months) or acute (single). Duodenal and rectal biopsies (5-6 per area) were stained using HEPS and evaluated by two boarded pathologists. Histomorphometry was used to count B-cells (CD20) and T-cells (CD3) using immunohistochemistry (absolute number per field). Data were analyzed using T-test or Mann-Whitney test.

Results: Twenty-one horses had acute colic and 39 had chronic colic. Gastrointestinal infiltrations were observed in 81% (+/- 8%) of acute colic horses and in 76.9% (+/- 7%) of chronic colic horses. Brunner's gland hyperplasia was significantly (p=0.011) more frequently observed in acute colic horses compared to chronic cases. In the rectum, there is a positive correlation between the lesion intensity observed on HEPS and the number of lymphocytes identified using IHC in the lamina propria (estimate = 1.98 [0.13; 3.84], p = 0.038) and the subcryptal area (estimate = 3.52 [0.02; 7.03], p = 0.049) respectively.

Conclusions: An inflammatory process in the gastrointestinal tract was identified in horses with acute colic and highlights the clinical relevance to perform biopsies in this specific context

Monday, November 18, 2024
10:57 AM – 11:04 AM

OTOCEPHALY: AGNATHIA- MICROSTOMIA-SYNOTIA SYNDROME IN TWO RHESUS MACAQUES (MACACA MULATTA)

Ahmad Saied[1], Krystal Vail[1], Adam Myers[1], Eric Vallender[1,2]
[1]Tulane National Primate Research Center, Covington, LA, USA, [2]University of Mississippi Medical Center, Jackson, MS, USA

Agnathia- Microstomia-Synotia Syndrome also known as otocephaly, is a very rare, lethal, congenital malformation characterized by: mandibular hypoplasia or agnathia, microstomia, aglossia, and ear anomalies (melotia/synotia). In humans, the syndrome develops at the 4th or 5th week of gestation due to failed migration of neural crest cells from the hindbrain. The failure of migration of these cells is linked to both genetic and teratogenic factors. Two, stillborn, male Rhesus Macaques (*Macaca mulatta*), presented for necropsy from the breeding colony at the TNPRC. Grossly, both animals manifested with synotia, microstomia, agnathia and aglossia/hypoglossia. Both animals belong to the same social group/enclosure, and they share the same sire. One animal had marked hydrocephalus and lissencephaly, with absence of olfactory bulbs. The brain of the other animal was grossly normal and had olfactory bulbs. Both animals had pulmonary atelectasis. Whole genome sequencing of bone marrow samples from both animals revealed that both animals are homozygous for a unique H197R mutation in the Twisted gastrulation BMP signaling modulator 1 gene (*TWSG1*). The affected animals are the result of separate half-avuncular matings of parents heterozygous for the mutation. *TWSG1* has multifaceted functions that include embryogenesis. Mutations in this gene have been shown to cause defects in osteogenesis and variable craniofacial phenotypes in knockout rodent models. Agnathia-Otocephaly complex is a very rare congenital anomaly in humans, and to our knowledge, this is the first report of this condition in non-human primates.

Monday, November 18, 2024
11:04 AM – 11:11 AM

OVARIAN SEX CORD TUMORS IN DOMESTIC SPECIES: 22 YEARS RETROSPECTIVE STUDY

Esdras Correa dos Santos[1], Doris Miller[1,2], Kaori Sakamoto[3], Elizabeth Howerth[1], K Paige Carmichael[1], Elizabeth Uhl[1], Daniel Rissi[1,2], Lilian De Jesus Oliveira[1]
[1]University of Georgia - College of Veterinary Medicine, ATHENS, GA, USA, [2]Athens Veterinary Diagnostic Laboratory - University of Georgia, ATHENS, GA, USA, [3]North Carolina State University, Raleigh, NC, USA

Background

Sex cord-stromal tumors in the ovary originate from constituents of the follicular endocrine apparatus. These tumors may arise from the theca cells, granulosa cells, or their luteinized derivatives. The classification of these tumors includes granulosa cell tumors, thecomas, luteomas, and mixed-pattern tumors.

Objective

This study aims to describe the type and frequency of ovarian sex cord tumors diagnosed in domestic species submitted to the Athens Veterinary Diagnostic Laboratory—University of Georgia, Athens, Georgia, United States (AVDL) from 2002-2024.

Methods

This study involved collecting and analyzing samples from necropsy and biopsy services at the AVDL, for over 22 years. Immunohistochemical (IHC) staining was employed to identify specific markers, such as inhibin-α and Melan-A, to aid the diagnosis.

Results

Over a span of 22 years, a total of 246 ovarian sex-cord origin tumors were identified across various domestic species. Among these, 243 were granulosa cell tumors, distributed as follows: 195 in canines, 32 in equines, 13 in felines, and 3 in caprines. Only eight of these tumors were classified as malignant, with 7 occurring in canines and 1 in a feline Additionally, other sex cord tumors included 2 luteoma (canine and feline) and 1 mixed-pattern tumor (equine). Although granulosa cell tumors were the most prevalent, they exhibited diverse morphological patterns within the same tumor, including solid sheets, cords, trabeculae, and nests.

Conclusions

Granulosa cell tumor is the most common tumor diagnosed. Most of the sex-cord tumors in this study were benign and occurred in canine species.

Monday, November 18, 2024
11:11 AM – 11:18 AM

UTILIZING A LARGE LANGUAGE MODEL FOR INFORMATION EXTRACTION FROM VETERINARY ELECTRONIC HEALTH RECORDS

Judit M Wulcan[1], Jonatan Wulcan[2], Kevin L Jacques[1], Mary Ann Lee[3], Samantha L Kovacs[1], Nicole Dausend[4], Lauren E Prince[4], Sina Marsilio[4], Stefan M Keller[1]
[1]University of California Davis, School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology, Davis, CA, USA, [2]Independent researcher, Malmoe, Sweden, [3]Colorado State University, College of Veterinary Medicine and Biomedical Sciences, James L. Voss Veterinary Teaching Hospital, Fort Collins, CO, USA, [4]University of California Davis, School of Veterinary Medicine, Department of Medicine and Epidemiology, Davis, CA, USA

Background: The lack of efficient methods for extracting information from free text in electronic health records (EHRs) poses a barrier to conducting large retrospective studies in veterinary pathology. Manual extraction is time-consuming, subjective, and error prone. Large language models (LLMs) can interpret and summarize free text, but their accuracy for information extraction from veterinary EHRs is uncertain.

Objective: This study evaluates the performance of a LLM in extracting information from veterinary EHRs compared to humans.

Methods: Using GPT4o (Open AI, San Francisco, USA) at temperature 0 and five humans, the presence of six clinical signs related to feline chronic enteropathy (FCE) was extracted from 250 randomly selected EHRs from the Veterinary Medical Teaching Hospital at the University of California, Davis.

Results: The LLM's performance compared to the majority opinion of the five humans, averaged across all six clinical signs, was as follows: Sensitivity: 97% (interquartile range [IQR] 93-99%), specificity: 98% (IQR 97-99%), positive predictive value: 81% (IQR 71-85%), negative predictive value: 100% (IQR 99-100%), F1 score: 84% (IQR 77-90%) and balanced accuracy: 96% (IQR 95-98%). Repeated LLM runs were more concordant than human assessor pairs (average Cohen's kappa 98 % (IQR 98-99 %) vs. 80% (IQR 78-81%), respectively). Most errors occurred in questions with human disagreement (35/43 [81%]).

Conclusion: Automated information extraction from veterinary EHRs using an LLM is a viable alternative to manual extraction by humans. This approach could enable more efficient, scalable, and objective analyses of datasets, facilitating integration of clinical data with pathology findings in retrospective studies.

Monday, November 18, 2024
11:18 AM – 11:25 AM

INSTAR, MICROSPORIDIAN, BACTERIAL, AND FUNGAL INFECTIONS IN A COHORT OF EASTERN LUBBER GRASSHOPPERS (ROMALEA MICROPTERA)

Naomi Falconnier[1], Adrien Izquierdo[2], Amira Ahmed[1], Luis Tondo[1], Jeongha Lee[1], Mariano Carossino[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]Audubon Nature Institute, New Orleans, LA, USA

Background: Eastern lubber grasshoppers (*Romalea microptera*) are native to the southeastern United States, and large numbers can result in economic citrus and vegetable crop losses. Control methods mainly consist of insecticides as natural predators are few.

Objective: The goal of this study was to characterize histological alterations associated with various infectious organisms in a cohort of adult lubbers.

Methods: Three days post-transfer, one of fifty adult lubbers was found dead with no premonitory signs. Following this, four clinically normal lubbers were euthanized for further investigation. All five adult lubbers were submitted for postmortem examination and were subjected to routine, Gram, and/or Fite-Faraco and Grocott-Gömöri's methenamine silver histochemical staining.

Results: Numerous instars (maggots) were mainly within abdominal tracheae of the naturally deceased lubber, with minimal associated inflammation. In all euthanized lubbers, gram-variable, weakly acid-fast microsporidians were within cytoplasmic perinuclear vacuoles of digestive cecal and midgut epithelium. Numerous gram-variable microsporidian spores were within the chitinous lined gut contents of all lubbers. The midgut and digestive ceca of the naturally deceased lubber were transmurally necrotic and melanized, with systemic hemocoagula, melanization, and associated gram-negative coccoid bacteria. Randomly distributed throughout the hemocoel of all euthanized lubbers were melanized hemocyte nodules, many of which were centered on fungal hyphae.

Conclusions: Findings in this cohort consisted of tracheal instar infestation, of undetermined identity; intestinal microsporidiosis, morphologically consistent with *Encephalitozoon romaleae;* and systemic bacterial and fungal infections. Although *E. romaleae* is considered highly contagious and fatal in several lubber species, some *R. microptera* colonies are reportedly asymptomatic.

Monday, November 18, 2024
11:25 AM – 11:32 AM

LYMPH NODE LIPOMATOSIS IN C57BL/6J MICE

Polly Chen[1], Jessica Wong[2], Rosalinda Doty[2], Marietta Barro[1], Pedro Ruivo[1], Denise Imai[1]
[1]Comparative Pathology Laboratory, School of Veterinary Medicine, University of California, Davis, Davis, CA, USA, [2]The Jackson Laboratory, Sacramento/Bar Harbor, CA, USA

Lymph node lipomatosisis is an age-related finding in older obese human patients, can cause lymphadenomegaly and is characterized by centrifugal replacement of lymphoid parenchyma by adipose tissue. This retrospective case series describes the presentation, diagnostic features and immunomorphology of lymph node lipomatosis in C57BL/6J mice. From 2013 – 2024, 14 experimentally naïve C57BL/6J mice were submitted for evaluation of large subcutaneous masses.

The masses occurred at sites where lymph nodes are expected, most commonly located at the ventral neck (n=11, 78.6%), followed by the inguinal region (n=2), axilla (n=1), and hilar region (n=1). Affected mice were predominantly female (n=12, 85.7%). Ages ranged from 4.4 to 19.7 months (median 10.5 months). The masses were smooth, encapsulated, tan to pink with prominent vasculature and had a firm fatty consistency. Histologically, the masses were composed of lymphofollicular structures surrounded and dissected by abundant adipose tissue and small muscular vessels lined with prominent endothelial cells. Immunohistochemistry targeting CD31, smooth muscle actin (SMA), CD3, CD19, and F4/80 revealed these follicular structures to be consistently characterized by central CD19-immunoreactive B cells with paracortical CD3-immunoreactive T cells and perifollicular F4/80-immunoreactive macrophages. SMA and CD31-immunoreactivity highlighted the density of small arterioles and identified small numbers of high endothelial venule-like vessels. Similar to human patients, lymph node lipomatosis in B6 mice can cause lymphadenomegaly and is associated with advanced age and female sex. In B6 mice, lymph node lipomatosis predominantly occurs in the cervical lymph node.

Monday, November 18, 2024
11:32 AM – 11:39 AM

RHABDOMYOSARCOMA IN A PREHENSILE-TAILED PORCUPINE (COENDOU PREHENSILIS)

Bayla Q. Bessemer[1], Megan E. Schreeg[1], Layne Staab[2], Jeff R. Hayes[1]
[1]The Ohio State University College of Veterinary Medicine department of Veterinary Biosciences, Columbus, OH, USA, [2]Boonshoft Museum of Discovery, Discovery Zoo, Dayton, OH, USA

A captive 4-yr-old female prehensile-tailed porcupine (Coendou prehensilis) had quill loss caudal to the right ear associated with a ~1-2 cm soft tissue mass effect. The mass was surgically debulked and described as a poorly differentiated neoplasm on histology. Ten days after surgery, the porcupine became anorectic and severely ataxic with a moderate left sided head tilt, and was euthanized and submitted for a postmortem examination. Deep to the surgical site was a 7x3x0.5cm amorphous, tan, soft, irregularly shaped mass that encircled the right ear canal and invaded skeletal muscle and the tympanic bulla. Histopathology revealed a hypercellular, infiltrative and destructive, unencapsulated neoplasm comprised of interlacing bundles, streams, and herringbone arrangements of spindle cells supported by a scant fine fibrovascular stroma. Cells were oval with indistinct cell margins, scant to moderate granular eosinophilic cytoplasm, and coarsely stippled chromatin with 1-3 nucleoli. Anisocytosis and anisokaryosis were moderate, with high mitotic activity and occasional multinucleated giant cells. Neoplastic cells invaded through adjacent myofibers and bone of the tympanic bullae immediately adjacent to nerves, consistent with antemortem neurologic signs. Neoplastic cells exhibited cytoplasmic immunolabeling for vimentin, smooth muscle actin (SMA), and desmin, and were immunonegative for S-100 and CD-31. The mass was diagnosed as a rhabdomyosarcoma. To our knowledge, this is the first diagnosis of a rhabdomyosarcoma in a prehensile-tailed porcupine. This case report expands the current knowledge of neoplasia in this species while providing an opportunity to validate immunohistochemistry in this species.

Monday, November 18, 2024
11:39 AM – 11:46 AM

CANINE KELOIDAL FIBROMA: A CYTOLOGICAL, HISTOLOGICAL AND IMMUNOHISTOCHEMICAL INVESTIGATION

Jasmine Chan, Marvin Firth, Stacey Newton
Nationwide Laboratories, Poulton-le-Fylde, United Kingdom

A 6-year-old male neutered Boxer was sampled with a history of a recent approximately 2 cm nodule adjacent to the left stifle. The primary practitioner submitted fine needle aspirates from the mass for cytology. Initial digital cytological features included predominantly a mild to moderately pleomorphic population of spindle cells with several clumps of eosinophilic amorphous material in thick strands as

well as more oval to elongated clumps that were mostly thickly spread. Further investigation with histology was advised due to the suspicion of a possible spindle cell tumor. Subsequent excisional biopsy and assessment using digital histopathology confirmed features consistent with a keloidal fibroma. This case also highlights immunohistochemical features of keloidal fibroma.

Keloidal fibroma is a rare tumor that has been recognised in dogs and humans but not documented in other animal species. Short-haired dog breeds including Boxers are most commonly affected. Males are predisposed in canines, which is the opposite seen in humans. The exact pathogenesis is unknown, but, in both human and dogs, they are considered to emerge from an inflammatory process. The vast majority of keloidal fibromas are benign and surgical excision is curative. This case aims to highlight the benefit of cytological and histological correlates in fibroproliferative lesions and reviews some of the classic features of this condition when examined digitally. Due to the possibility of malignant transformation into keloidal fibrosarcomas, early and accurate diagnosis is encouraged with this condition.

Monday, November 18, 2024
11:46 AM – 11:53 AM

COMPARATIVE PATHOLOGY OF CHOLANGIOCARCINOMA ACROSS SPECIES: INSIGHTS FROM VETERINARY AND HUMAN MEDICINE

Katti Crakes[1], Hannah Stephen[1,2], Amanda Maxwell[1], Kiyoko Oshima[1], John Trupkiewicz[1], Cory Brayton[1]
[1]Johns Hopkins University, School of Medicine, Baltimore, MD, USA, [2]University of Georgia, College of Veterinary Medicine, Athens, GA, USA

Cholangiocarcinoma (CCA) is an aggressive hepatobiliary neoplasm that affects a wide range of species, including humans, dogs, cats, bears, ruminants, birds, and reptiles. Despite being the second most common primary liver tumor in humans, veterinary literature on CCA remains limited to sporadic case reports primarily in companion animals. This study aims to classify gross and histologic subtypes of CCA in veterinary species, drawing parallels with human medicine.

We examined 18 cases of CCA in companion animals, laboratory animals, and zoo/wildlife (mammals, n=9; reptiles, n=6; avian, n=3) submitted to Johns Hopkins Comparative Pathology from 1986-2024. Each case was reviewed for gross and histologic classification based on location (intrahepatic or extrahepatic), gross subtype (mass-forming, perihilar, or intraductal), neoplastic origin (small or large duct), and differentiation (well, moderate, or poor). The majority of cases were intrahepatic and mass-forming (16/18), with neoplastic origins in both large (10/18) and small ducts (8/18). Differentiation varied, with well (9/18), moderate (7/18) and poorly (2/18) differentiated tumors. Metastatic spread was observed in 5/18 cases, involving the spleen, intestines, lung, heart, pancreas, kidney, and mesentery.

Immunohistochemical staining identified CK7 and 19 are identified as reliable biliary markers, whereas CK20 yielded equivocal results. Additionally, six cases initially resembling CCA were reclassified as benign biliary neoplasms, neuroendocrine tumors, polycystic liver disease, and metastatic adenocarcinoma. Finally, we summarize the diagnostic criteria and nomenclature between human (WHO) and veterinary (WSAVA, INHAND) guidelines, underscoring the need for uniformity in a comparative approach to advance our understanding of translational models and clinical treatment of CCAs.

Experimental Disease Abstracts

Chair: Manu Sebastian

Monday, November 18, 2024
02:15 PM – 02:30 PM

PRECLINICAL DOG MODEL OF FOCAL PROSTATE CANCER: PATHOLOGY AND NOVEL THERANOSTICS

Nathan Hoggard[1], Ramamurthy Gopalakrishnan[2], Felipe Berg[2,3], Xinning Wang[2], Eric Hosnik[4], Matthew Joseph[5], Reena Shakya[5], Krishan Kumar[5], Richard James[5], Arijit Ghosh[5], Dong Luo[6], Dario Palmieri[7], Michael Knopp[5], Agata Exner[2], James Basilion[2], Michael Tweedle[5], Thomas Rosol[1]
[1]Heritage College of Osteopathic Medicine, Ohio University, Athens, OH, USA, [2]College of Medicine, Case Western Reserve University, Cleveland, OH, USA, [3]Hospital Israelita Albert Einstein, Sao Paulo, Brazil, [4]College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA, [5]College of Medicine and the Wright Center of Innovation in Biomedical Imaging, The Ohio State University, Columbus, OH, USA, [6]College of Engineering, South China University of Technology, Guangzhou, China, [7]Cancer Treatment and Research Center, The Ohio State University, Columbus, OH, USA

Introduction: Dogs develop prostate cancer (PCa) similar to men. We refined a beagle model of PCa using canine Ace-1 cells. The pathology and theranostic (therapeutic-diagnostic) applications are described. **Methods:** Prostates of immunosuppressed, intact, beagle dogs (n=30) were inoculated with Ace-1 cells stably transduced with human gastrin-releasing peptide receptor (hGrPR; n=20), human prostate-specific membrane antigen (hPSMA; n=9), or canine vascular endothelial growth factor A (cVEGFA; n=1). Theranostics were injected into the prostatic artery with fluoroscopic guidance: 1) bombesin peptide analogs (BBN) for hGrPR conjugated with an 800nm near-infrared (NIR) fluorescent dye (n=20) or Lutetium-177-labeled-chelate (n=1) and 2) gold nanoparticles conjugated with a hPSMA ligand and NIR dye (for photodynamic therapy, PDT). All dogs were euthanized 5 weeks after tumor inoculation. Prostate glands and Ace-1^{hPSMA} tumors were processed for histopathology, immunohistochemistry, and quantitative PCR. **Results:** The model reliably formed intraprostatic and regionally invasive capsular/intraperitoneal tumors (0.5-2cm). Intraprostatic tumors were within pre-existing glands, had periglandular inflammation, were slow growing, and had low Ki-67 immunolabeling. Regionally invasive tumors grew into and through the capsule, induced granulation tissue, had higher Ki-67 staining ($p<0.01$), upregulated targetable growth factor receptors (*Pdgfra, Slc2A1*), and downregulated angiogenesis genes (*Vegfa*, *Vegfc*, and their receptors). BBN-NIR perfused the prostate and bound specifically to PCa within 2h. Systemic IV PDT demonstrated internalization of the hPSMA ligand in the tumors. The tumors were treated with a 700 nm laser that induced superficial tumor necrosis. **Conclusion/Impact:** We demonstrated refinement of the dog model of PCa with proof-of-principle for novel therapies/diagnostics.

Monday, November 18, 2024
02:30 PM – 02:45 PM

IMPACTS OF ARTIFICIAL SWEETENERS ON THE GUT MICROBIOME IN THE PATHOGENESIS OF METABOLIC SYNDROME

Katti Crakes[1,2], Alex Crits-Christoph[1], Lauren Questell[1], Subah Soni[1], Valerie Harrington[1], Lilian Lau[1], Lincoln Hopkins[1], Jiaheng Huang[1], Courtney Dunning[1], Emily Wissel[1], Haley Hallowell[1], Hila Winer-Yanai[1], Cory Brayton[2], Jotham Suez[1]
[1]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD, USA, [2]Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Non-caloric artificial sweeteners (AS) are commonly used to replace dietary sugars in the prevention and treatment of metabolic syndrome. The efficacy of this approach has been challenged, and some studies suggest that AS may counterintuitively promote the same conditions they aim to treat. Additional evidence for causality and a better understanding of the underlying mechanisms are required to develop data-driven recommendations for AS consumption. Our research in preclinical models and human trials demonstrates that AS are not inert to the gut microbiome and causally link

microbiome alterations by AS to impaired metabolic health. We further show that the impacts of AS on metabolic health are person-specific and microbiome-dependent, which may explain the heterogeneity in trial outcomes and guide future clinical assessments.

To uncover the mechanisms through which AS impact the gut microbiome, we are conducting a high-throughput screen of diverse gut microbial communities and monocultures exposed to AS under anaerobic conditions, coupled with metagenomics and transcriptomics. We demonstrate that AS directly impact microbiome composition and function as well as gut bacterial physiology and bacterial growth in a dose-, sweetener-, and species-dependent manner. Notably, transplanting germ-free mice with AS-modulated microbiomes was sufficient to impair glucose tolerance and exacerbate weight gain, along with epididymal white adipose tissue adipocyte hypertrophy and liver inflammation. Furthermore, the AS-altered microbiomes significantly altered the hepatic transcriptome and the fecal metabolome. Collectively, our unbiased, multi-omics approach uncovered several pathways through which the gut microbiome can potentially mediate the personalized impacts of AS on metabolic health.

Monday, November 18, 2024
02:45 PM – 03:00 PM

DEXAMETHASONE REDUCES PULMONARY PATHOLOGY BUT DOES NOT ALTER MORTALITY IN SYRIAN HAMSTERS INFECTED WITH NIPAH VIRUS

Kerry Goldin, Tessa Lutterman, Brandi Williamson, Manmeet Singh, Emmie de Wit
Laboratory of Virology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Nipah virus (NiV) is a zoonotic pathogen that causes severe respiratory and neurologic disease in humans, and mortality is typically very high (~70%). In cases of severe COVID-19, treatment with dexamethasone at an advanced state of disease improved clinical outcomes. In this study, we determined the effect of dexamethasone treatment on Syrian hamsters infected with NiV. Syrian hamsters were treated with an anti-inflammatory dose of dexamethasone for 10 days, starting at 4 days post infection. Dexamethasone treatment did not result in differences in virus shedding from the nose or throat, viral loads in tissues, or cytokine levels in the lungs. However, animals treated with dexamethasone had marked reduction in pulmonary pathology, compared to animals in the PBS-treated groups. In PBS-treated animals common lung findings included marked pulmonary edema and fibrin exudation, interstitial pneumonia, and vasculitis with fibrin thrombi formation and rare endothelial syncytia. Surprisingly, the reduction in pulmonary pathology observed with dexamethasone treatment did not result in changes in disease onset, nor in a reduced mortality rate. Our findings suggest that pulmonary pathology isn't the sole driver of mortality in cases of NiV infection. In human cases of NiV infection, where supportive care can be administered, the absence of pulmonary lesions may be of clinical benefit. Next, we will combine dexamethasone with the antiviral drug, remdesivir, to determine if combined treatment improves outcome compared to treatment with either drug on its own. This work was supported by the Intramural Research Program of NIAID, NIH.

Monday, November 18, 2024
03:30 PM – 03:45 PM

EXPERIMENTAL INFECTION OF WILD TURKEYS WITH LYMPHOPROLIFERATIVE DISEASE VIRUS

Chloe Goodwin[1], Jian Zhang[1], Andrew Allison[2], Rebecca Poulson[1], Mark Ruder[1], Paul Oesterle[1], Elizabeth Kurimo-Beechuk[1], Nicole Nemeth[1]
[1]University of Georgia, Athens, GA, USA, [2]University of Florida, Gainesville, FL, USA

Background: Wild turkeys are frequently infected with lymphoproliferative disease virus (LPDV), an oncogenic virus that can induce subclinical infection to multicentric lymphoma. Despite its widespread occurrence and high prevalence across much of North America, pathogenesis is poorly understood.

Objective: To induce experimental infection of wild turkeys with LPDV and evaluate clinical behavior, hematology, gross and microscopic pathology, and viral DNA shedding for up to 6 months.

Methods: Eggs were collected from abandoned nests, incubated, hatched, and captive reared. Twenty-four approximately 3-month-old wild turkeys were inoculated with serum from experimentally infected domestic turkeys via subcutaneous injection (n=18) or skin scarification (n=6); six turkeys served as contact controls. Behavior was observed remotely (via camera) 3 times/week. Blood (whole blood, blood smears, serum) and swabs (oropharyngeal, cloacal) were collected weekly. The turkeys were euthanized at 3-months or 6-months post-inoculation. Select tissues were evaluated via PCR, histopathology, and in situ hybridization (ISH).

Results: Most (23/24; 96%) inoculated turkeys had evidence of LPDV infection, along with 3/6 (50%) contact controls. Oropharyngeal and cloacal swabs were persistently tested positive, while blood was slightly less frequently positive. One turkey developed paraplegia, wing droop, and seizures at 45 days post-inoculation due to lymphoma in brain and spinal cord. Other infected turkeys exhibited variable diminished to increased activity levels. Antemortem samples from one turkey consistently tested PCR-negative, but infection was evident via PCR and ISH of tissues. Most tissues from infected birds tested PCR-positive and exhibited lymphocytic infiltration. Five turkeys developed multi-organ lymphoma and lymphocytic foci exhibited ISH labeling.

Monday, November 18, 2024
03:45 PM – 04:00 PM

EVALUATING MICRORNA-29 REDUCTION IN THE BRAINS OF ALZHEIMER'S DISEASE AND WILD TYPE MOUSE MODELS

Jonathan Nagel, Nazanin Kiapour, Chloe Deshusses, Keeley Spiess, Mohanish Deshmukh
University of North Carolina, Chapel Hill, NC, USA

Background: MicroRNA-29 (miR-29) is vital to mature brain homeostasis, and reduced levels of miR-29 in the brain have been associated with Alzheimer's disease (AD). While miR-29 offers a potential avenue of AD therapy, the effects of miR-29 reduction in the brain are not entirely understood.

Objectives: We aim to: 1) Define the changes in miR-29 levels in multiple murine models of AD; 2) Evaluate the effects of reduced miR-29 in the neurons of adult wild type mice.

Methods: 1) We measured brain miR-29 levels (qRT-PCR) in three murine AD models: APP/PS1, PS19, N-L-GF; 2) We created a cre/lox mouse model to induce complete (miR-29 F/F) or partial (miR-29 F/wt) deletion of miR-29 in Thy1-positive neurons at 4 months of age. These mice were evaluated for survivability, gene expression changes, behavioral abnormalities, and AD-associated lesions.

Results: 1) Brain miR-29 was reduced by 10-40% in all three AD murine models evaluated; 2) While partial deletion of miR-29 did not affect survivability, complete deletion resulted in premature lethality. Deletion of miR-29 also resulted in altered expression of several AD-associated genes (e.g. *NAV3*).

Conclusions: 1) miR-29 levels are reduced in several established AD mouse models; 2) miR-29 expression in neurons is vital to brain homeostasis, and reduced miR-29 likely plays a role in AD pathogenesis.

Impact Statement: The links between miR-29 and AD shown here further support miR-29 as a promising AD treatment. Future directions include evaluation of AAV gene therapy to restore endogenous levels of miR-29 in the brains of AD model mice.

Monday, November 18, 2024
04:00 PM – 04:15 PM

CATTLE WITH THE EK211 PRNP POLYMORPHISM ARE SUSCEPTIBLE TO THE H-TYPE BOVINE SPONGIFORM ENCEPHALOPATHY AGENT FROM EITHER E211K OR WILD TYPE DONORS AFTER ORONASAL INOCULATION

Justin Greenlee[1], Eric Cassmann[1], S. Jo Moore[1,2], M Heather West Greenlee[3]
[1]Virus and Prion Research Unit, National Animal Disease Center, ARS, United States Department of Agriculture, Ames, IA, USA, [2]Oak Ridge Institute for Science and Education (ORISE), U.S. Department of Energy, Oak Ridge, TN, USA, [3]Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA

In 2006, a case of H-type bovine spongiform encephalopathy (H-BSE) was reported in a cow with a previously unreported prion protein polymorphism (E211K). The E211K polymorphism is heritable and homologous to the E200K mutation in humans that is the most frequent *PRNP* mutation associated with familial Creutzfeldt-Jakob disease. The purpose of this study was to investigate whether the agent of H-type BSE transmits to wild type cattle or E211K cattle after oronasal exposure. Wild type (EE211) or heterozygous (EK211) cattle were orally inoculated with the H-BSE agent from either the US 2004 case (wild type donor; n=3) or from the US 2006 (E211K donor; n=4). Cattle were necropsied when clinical signs of prion disease were observed, or at the end of the experiment (84 months). Cattle were confirmed positive for BSE prions by enzyme immunoassay and anti-PrP immunohistochemistry (IHC) on brainstem. Three-out-of-four (75%) calves with the EK211 genotype developed clinical signs of H-BSE including inattentiveness, loss of body condition, weakness, ataxia, and muscle fasciculations and were euthanized. Two of the positive EK211 steers received H-BSE US 2004 inoculum (Incubation Period (IP): 59.3 and 72.3 months) while the other positive steer received the E211K H-BSE inoculum (IP: 49.7 months). All wild type recipient cattle and a single EK211 steer remained asymptomatic for the duration of the experiment and no abnormal prion protein was detected in these cattle by EIA. This study demonstrates that the H-type BSE agent is transmissible by the oronasal route to cattle with the EK211 *PRNP* genotype.

Monday, November 18, 2024
04:15 PM – 04:30 PM

CHLAMYDIA MURIDARUM CAUSES PERSISTENT INFECTION AND INDUCES SUSTAINED INTESTINAL T-CELL AND ILC3 RESPONSES IN INBRED AND OUTBRED MICE FOLLOWING THE PRESUMPTIVE ROUTE OF NATURAL INFECTION

Sebastian E. Carrasco[1,2], Noah Mishkin[2], Michael Palillo[2], Panagiota Momtsios[3], Cheryl Woods[3], Kenneth Henderson[3], Ana Leda F Longhini[4], Chelsea Otis[4], Rui Gardner[4], Ann M Joseph[5], Gregory F Sonnenberg[5], Jack Palillo[6], Rodolfo Ricart Arbona[2], Neil Lipman[1,2]
[1]Laboratory of Comparative Pathology, Weill Cornell Medicine, Memorial Sloan Kettering Cancer Center, The Rockefeller University, New York, NY, USA, [2]Center for Comparative Medicine and Pathology, Memorial Sloan Kettering Cancer Center and Weill Cornell Medicine, New York, NY, USA, [3]Research Animal Diagnostic Services, Charles River Laboratories, Wilmington, MA, USA, [4]Flow Cytometry Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [5]Weill Cornell Department of Medicine, Weill Cornell Medicine, New York, NY, USA, [6]Neurologic Clinical Research Institute, Massachusetts General Hospital, Boston, MA, USA

Chlamydia muridarum (Cm) was recently rediscovered in research mouse colonies. Our group has shown that natural Cm infections are associated with disease and pathology in select immunodeficient mouse strains. This study evaluated the immunopathological impact of Cm infection

on commonly utilized immunocompetent C57BL/6J (B6), BALB/c (C), and J:ARC(S) mice. A Cm field isolate was administered (orogastric gavage) to 8-week-old female C mice. After confirming shedding, these mice were cohoused with naïve B6, C, and S mice (n=28/strain) for 30 days. Three-to-six mice were evaluated 7, 14, 21, 63, 120, and 180 days post-cohousing (DPC) via hemograms, serum biochemistry, fecal qPCR, necropsy, histopathology, and Cm MOMP immunohistochemistry. Immunophenotyping was performed on intestines from B6 mice at 14 and 63 DPC. All strains were shedding Cm by 28 through 180 DPC. No cohoused Cm-infected mice developed clinical signs, gross lesions or had abnormalities in CBC and serum biochemistry analytes throughout the study. GALT hyperplasia was frequently noted in numerous infected mice. Cm antigen was often detected in enterocytes overlying GALT. A subset of B6 (10/18) and S (7/18) mice developed a minimal-to-mild typhlocolitis. Intestinal immunophenotyping of the Cm-infected B6 mice demonstrated sustained increases in neutrophils, Th1 and Th17 cells, and group 3 innate lymphoid cells (ILC3) as compared to the controls. Collectively, these results demonstrate that while no clinical disease was appreciated in these mouse strains, Cm likely causes a life-long infection with the potential to induce a significant induction of myeloid cells, increase pro-inflammatory cytokines and activate T-cell and ILC subsets.

Monday, November 18, 2024
04:30 PM – 04:45 PM

PROGRESSION OF VIRAL REPLICATION AND LESIONS IN MOUSE KIDNEY PARVOVIRUS INFECTION IN CD1, B6, AND NSG MICE

Ileana Miranda[1], Mandy Kain[1], Amanda Ritter[2], Neil Lipman[2], Sebastien Monette[1]
[1]Memorial Sloan Kettering Cancer Center, Weill Cornell Medicine, and The Rockefeller University, New York, NY, USA, [2]Memorial Sloan Kettering Cancer Center and Weill Cornell Medicine, New York, NY, USA

Background: Mouse kidney parvovirus (MKPV) causes chronic kidney disease resulting in morbidity and mortality in immunodeficent mice and subclinical infection in immunocompetent mice. While chronic renal lesions and viral replication have been characterized, a multisystemic investigation of MKPV infection from the acute to the chronic stages of infection has not been described.

Objective: To investigate lesions and viral replication in all major organs at all stages of MKPV infection in immunocompetent Crl:CD1(ICR) (CD1) and C57BL/6NCrl (B6), and immunodeficient NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice.

Methods: Following experimental oronasal inoculation with MKPV, mice were evaluated at 20 time points from 1.5 to 168 days by histopathology and MKPV RNA *in situ* hybridization (ISH) on all major organs, and immunohistochemistry for markers of immune cells and renal tubular injury on kidneys.

Results: In all strains, gastrointestinal replication was observed by day 3 and persisted throughout the course of the study but was not associated with lesions. In CD1 and B6 mice, replication was first detected in renal tubules at days 14 and 28, respectively, in association with lymphocytic tubulointerstitial nephritis first evident at days 49 and 63. In NSG mice, viral replication in the kidneys was first detected at day 42 and associated tubular degeneration was evident from day 63. No replication or lesions were observed in other organs.

Conclusions: The gastrointestinal tract is the initial site of viral replication in MKPV infection in mice. In the kidneys, strain differences in the progression of replication and lesions are observed in B6, CD1, and NSG mice.

Monday, November 18, 2024
04:45 PM – 05:00 PM

CHARACTERIZATION OF A NOVEL N-NITROSO-N-METHYLUREA (NMU)-INDUCED TUMOR IN SIBERIAN HAMSTERS (PHODOPUS SUNGORUS)

José Vilches-Moure, Ching-Hsuan Chen, Nathan Fisher, Nirk Quispe-Calla, Norman Ruby
Stanford University School of Medicine, Stanford, CA, USA

Background: In females, breast cancer is the most commonly diagnosed malignancy. Those who work night shifts are 2.34 times more likely to develop this malignancy, due in part to circadian disruption. The Siberian hamster (*Phodopus sungorus*) has been used for circadian research due to the ease with which it can be rendered arrhythmic.

Methods: Siberian hamsters from a closed colony were injected with N-nitroso-N-methylurea (NMU), tumors were evaluated, and neoplastic cell cultures generated. Neoplastic cell cultures were passaged and later injected into additional animals, and the resulting solid tumors were evaluated.

Results: Tumors generated from direct NMU injection and from injected cell cultures have a similar mesenchymal morphology and locally invasive behavior. Tumors are arranged in interweaving bundles, are supported by varying amounts of fibrovascular stroma, and occasionally have areas of necrosis. Cells are spindle to polyhedral, exhibit prominent anisocytosis and anisokaryosis, occasional multinucleation, and mitotic figures that are sometimes bizarre. Cells have infrequent and patchy positive immunoreactivity for smooth muscle actin, pan-cytokeratin, cytokeratin 14, and less frequently to pan-muscle actin. Cells are generally negative for progesterone receptor (PR) and desmin.

Conclusions: Taking together the morphology and molecular profile of these tumor cells, we propose they may arise from the mammary myoepithelial cell, and these tumors may represent a chemically induced malignant myoepithelioma. This novel chemically induced tumor in Siberian hamsters may serve as a new tool in which to study the effects of circadian disruption on neoplastic transformation, development, and progression.

Industrial and Toxicologic Pathology

Chair: Caitlin Brown

Tuesday, November 19, 2024
02:15 PM – 02:30 PM

OPTIMIZATION OF IN VITRO CHEMORADIATION RESPONSE ASSAY IN CANINE BLADDER CANCER ORGANOIDS TO ASSESS FOR SYNERGISTIC ACTIVITY

Mackenzie Long[1], Hannah Nicholson[1], Michael Catucci[1], Bryan Melvin[1], Christopher Zdyrski[1], Aleksandra Pawlak[1,2], Corey Saba[1], Travis Laver[1], Emily Rawlings[1], Maria Orbay-Cerrato[1], Mohamed Elbadawy[1], Koichi Nagata[1], Karin Allenspach[1], Jonathan Mochel[1]
[1]University of Georgia College of Veterinary Medicine, Athens, GA, USA, [2]Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

Background: The prediction of treatment outcomes for high-grade muscle-invasive urothelial carcinoma (InvUC) is currently limited to traditional 2D-cell culture systems and mouse models. However, these do not accurately recapitulate the heterogeneity and biological behavior of tumors. Canine UC closely resembles human InvUC histologically, molecularly, and biologically; therefore, making it a relevant animal disease model. Additionally, 3D tumor organoid cultures have been shown to be more predictive than 2D-cell culture systems. As such, canine-derived 3D-organoid UC models could provide a relevant *in vitro* model to predict treatment responses. In both species, cisplatin is considered one of the most effective chemotherapeutics. Recent efforts have focused on

incorporating radiation therapy in combination with traditional chemotherapy to enhance therapeutic efficacy. **Objective:** To develop a robust screening assay for assessing chemoradiation and monotherapy response in urine- and tissue-derived canine UC organoid models. **Methods:** Four canine UC adult stem cell-derived organoids from urine or biopsies, were dissociated, plated (10,000 cells/well), and irradiated (5, 9, or 15 Gy) after 24 hours. After 72 hours of incubation, the organoids were exposed to cisplatin (10, 25, 50, or 100 µM). Untreated cells served as a control. After one week, metabolic activity (Presto Blue), apoptosis (Caspase 3/7 activation), and cell proliferation (Ki-67) were measured. **Results:** Chemoradiation induced greater cytotoxicity than monotherapy and a differential response was noted across patients (IC50: ranging from 10-50 µM; 5-9 Gy). **Conclusion:** This assay can be used to assess and compare monotherapy to chemoradiation response in canine urine- and tissue-derived organoids.

Tuesday, November 19, 2024
02:30 PM – 02:45 PM

VASCULAR PATHOLOGY ASSOCIATED WITH INDWELLING INTRAVENOUS CATHETERS IN SPRAGUE DAWLEY RATS: RECOMMENDED STANDARDIZED NOMENCLATURE AND FACTORS AFFECTING CATHETER PATENCY

Marc Mattix, David Rehagan, EJ Ehrhart, Michelle Magagna, Loni Schumacher, Teresa Gleason
Charles River Laboratories, Wilmington, MA, USA

Transcutaneous buttons are a proven refinement for intravenous (IV) infusion and blood sampling compared to traditional vascular access ports by reducing pain and distress from repeat needle punctures. However, catheter patency and vascular changes at the catheter tip present challenges in study conduct and interpretation of procedural versus test article-related microscopic findings. Factors influencing both catheter patency and microscopic vascular changes include duration of catheterization; catheter material, size, and style; catheter placement; and use of anticoagulant locking solutions. The effects of catheter style, venous placement, and locking solution on bidirectional catheter patency were evaluated in an 8-week study in Sprague Dawley rats surgically implanted with catheters attached to Vascular Access Buttons (VAB). In addition, procedure-related histologic changes at the catheter tip site were evaluated and recorded based on current International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) terminology. Background vascular lesions at the catheter tip consisted of intimal proliferation; medial thickening; thrombus; vascular degeneration/necrosis, necrosis/inflammation, or inflammation; perivascular fibrosis; hemorrhage; mineralization; and bacterial colonies. The use of standardized nomenclature to record catheter-associated vascular changes serves to provide consistency between studies and a framework to assist in differentiating procedural from test article-related vascular effects.

Tuesday, November 19, 2024
02:45 PM – 03:00 PM

DESCRIBING VISTA 360; AN INTERNAL PLATFORM FOR AI ASSISTANCE AND ALGORITHM INTEGRATION DURING PATHOLOGIST SLIDE REVIEW

Philip Zehnder, Ruth Sullivan, Reina Fuji, Fangyao Hu, Ben Freiberg
Genentech, South San Francisco, CA, USA

We have previously developed VISTA, an internal viewer system for pathologist review of Whole Slide Images (WSI) built on our FAIR data whole-slide image data repository. Here we report our extension of this infrastructure to incorporate VISTA360, a multi-modal dashboard based on an internal proof of concept (POC), which integrates a wide range of computational outputs and algorithm results to guide and inform pathologist slide assessment. Guided by our initial POC dashboard evaluation, we have incorporated a number of capabilities in VISTA360. Features include heat map overlays and visualization of high-lesion-probability tiles to support pathologist review of results of cutting edge AI/DL algorithms developed by our team to detect and classify lesions. Such

approaches currently comprise multiple out-of-distribution methods to agnostically detect anomalies on WSI, and MIL approaches to classify lesion types. In addition to these image-based visualizations, we include multiple graphical visualization approaches to display a broad array of numerical data outputs. Together, these capabilities support use of the menu of AI/DL algorithms developed by our team to enhance and extend pathologist slide review. Ultimately, VISTA360 will enable seamless use of computational approaches ranging from lesion-probability guided slide review to numerical output informed tissue interpretation into standard pathology practice in our group.

Tuesday, November 19, 2024
03:30 PM – 03:45 PM

A SPONTANEOUS MODEL FOR IGM GLOMERULONEPHROPATHY IN ATHYMIC NUDE MICE

Marietta Barro[1], Qian Chen[2], Anibal Armien[3,4], Laurie Brignolo[5,6], Denise Imai-Leonard[1,4]
[1]Comparative Pathology Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, [2]Center for Genomic Pathology Laboratory, School of Medicine, University of California-Davis, Sacramento, CA, USA, [3]California Animal Health & Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, [4]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, [5]Research and Teaching Animal Care Program, Office of Research, University California-Davis, Davis, CA, USA, [6]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA

Background and Objectives: Hsd:Athymic nude-*Foxn1*^{nu} mice, used as sentinels, spontaneously develop a life-limiting progressive protein-losing nephropathy. We aimed to characterize the disease, determine the prevalence and identify risk factors.

Methods and Results: Grossly, affected mice presented with anasarca and pale tan, irregularly pitted kidneys. Histologically, glomeruli were distorted by amorphous eosinophilic mesangial deposits, synechiae, periglomerular fibrosis and capillary thrombosis. Tubules were ectatic with attenuated epithelium and contained protein casts. Mesangial deposits were not congophilic, dark magenta with PAS, pale pink to light blue with Masson's trichrome, and the basement membrane appeared thickened with Jone's *methenamine* silver. The mesangial deposits were IgG and IgA-immunonegative and IgM-immunopositive. Ultrastructurally, deposits were composed of mixtures of straight or curved linear, 30–170 nm-diameter tubular, and 9–16 nm-diameter, fibrillary profiles. Vivarium biosecurity level, infectious disease screening results and the presence of co-morbidities were evaluated in 75 athymic nude sentinel mice with protein-losing nephropathy and 75 without. All the mice were 6-month-old homozygous females. Neither sex, age, biosecurity level, *Helicobacter* sp., Mouse norovirus, Murine chapparvovirus, nor total pathogen load was a risk factor. Auto-inflammatory conditions (proliferative typholocolitis, $p = 0.005$; dermatitis, $p = 0.03$) and neoplasia (lymphoma, $p = 0.0007$) had a protective effect, with lower risk of developing hyaline glomerulopathy.

Conclusion: Our results support the diagnosis of hyaline glomerulopathy in nude sentinel mice and propose that this could be a spontaneous model for human IgM nephropathy.

Tuesday, November 19, 2024
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MANAGING WSI QUALITY CONTROL WHILE BUILDING A VAST DIGITAL PATHOLOGY IMAGE REPOSITORY

Cyrus Manuel, Andy Cheon, Trung Nguyen, Fangyao Hu, Ruth Sullivan, Reina Fuji
Genentech, SOUTH SAN FRANCISCO, CA, USA

In 2019, our team undertook the digital scanning of glass histology slides from all our prospective animal studies and our historical slide-archive to convert our archive to a Whole Slide Image (WSI)

repository for digital pathology. In total, we aimed to scan hundreds of thousands of glass slides. To be fully useful for downstream digital pathology applications, such as developing AI/DL algorithms to enhance pathology practice, the WSI in the repository needs to be of high quality. In particular, out-of-focus scanning artifacts are deleterious both to pathologist review and interpretation of WSI and AI/DL or image-analysis algorithm development. In order to meet the need to ensure the high-quality of the WSI focus while constrained to existing staff to perform manual WSI review, we developed an in-house approach to incorporate computational support tools to massively speed the review process. Our approach utilizes a pair of models which we have customized to our context and integrated into our workflows. This approach saves hours of technician time and has enabled our team to keep up with WSI review demand throughout our repository building project without the addition of new staff.

Natural Disease Abstracts

Chair: Elena Alina Demeter

Monday, November 18, 2024
02:30 PM – 02:45 PM

OUTBREAK OF LETHAL CANINE DISTEMPER VIRUS INFECTION IN FREE-RANGING BLACK TUFFED MARMOSETS (CALLITHRIX PENICILLATA)

Daniel Santos[1], Bruna Campos[1], Lucas Souza[1], Nayara Paula[1], Andre Vieira[1], Leticia Ribeiro[1], Caio Figueiredo[1], Vinicius Amaral[1], Janaina Duarte[1], Carlyle Coelho[2], Herlandes Tinoco[2], Sara Santos[3], Felipe Iani[3], Erica Costa[1], Ayisa Oliveira[1], Marcelo Carvalho[1], Renato Santos[1]
[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]BH Zoo - Fundação de Parques Municipais e Zoobotânica, Belo Horizonte, Brazil, [3]Fundação Ezequiel Dias, Belo Horizonte, Brazil

Canine distemper virus (CDV) infections have been diagnosed in several wild mammalian species. Over a period of 39 days, seven adult free-ranging black tuffed marmosets (*Callithrix penicillata*) were found dead or captured at the Belo Horizonte Zoo. Two animals were apathetic before death, and two of them had neurological signs including myoclonus. Grossly, four animals had marked skin thickening with crusts, mostly evident on the face, thoracic limbs and the inguinal area. Histologically, skin lesions consisted of variable degrees of acanthosis (5/7), hyperkeratosis (6/7), degeneration (3/7), syncytial epithelial cells (5/7) and mild neutrophilic dermatitis (4/7). Brain lesions included gliosis (2/6) with gemistocytes (2/6) and Alzheimer type II cells (3/6), neuronal necrosis (3/6), meningitis (3/6), choroiditis (1/6), with intranuclear and intracytoplasmatic eosinophilic inclusion bodies in neurons and astrocytes (1/6). Syncytial cells were also observed in the epithelium of the tongue (6/7), parathyroid (3/3), and pituitary gland (1/7). Oral swabs were positive for CDV and negative for measles virus by RT-PCR. Immunohistochemistry (IHC) using a monoclonal antibody against CDV demonstrated viral antigens in the brain, skin, tongue, and lungs from all seven animals. Immunostaining was more intense in the frontal lobe and telencephalic cortex, but positive cells were also found in the occipital lobe, cerebellum, and brain stem. Other IHC-positive organs included liver (2/2), gall bladder (1/1), trachea (4/4), urinary bladder (1/1), testis (2/2), thyroid (5/5), and parathyroid (2/2). This is the first report of natural CDV infection in free-ranging neotropical primates, which may have major implications under an One Health perspective.

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PCV3-ABORTED FETUSES IN BRAZIL: CHARACTERIZATION OF PCV3-RNA DISTRIBUTION AND ASSOCIATED INFLAMMATION

Eryca Ceolin Lamego[1,2], Milena Carolina Paz[1], Paula Reis Pereira[1], David Driemeier[1], Lilian de Jesus Oliveira[2]
[1]Department of Pathology, Faculdade de Medicina Veterinaria, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [2]Department of Pathology, University of Georgia, Athens, GA, USA

Background Porcine-circovirus-type-3 (PCV3) infection has been linked with abortions and offspring that fail to thrive in pigs. Intrauterine infection yields weak offspring occasionally with dorsally rotated pinna (a.k.a. “Dumbo-like ears”) observed by farm workers.

Objective This study aimed to describe the lesions and characterize the PCV3 RNA distribution and phenotype of the inflammation in cases of PCV3-associated aborted fetuses in two farms located in the state of Santa Catarina, Brazil.

Methods Formalin-fixed tissues from seven aborted fetuses were evaluated for histologic lesions, in-situ hybridization for PCV3-RNA, and immunohistochemistry for T-cell (CD3), B-cell (CD20), and macrophages (IBA1).

Results In 4 out of 7 fetuses, PCV3-RNA was detected within the vascular wall, perivascular of the lungs, liver, kidney, spleen, intestines, testis, pinna, and mesentery. PCV3-associated perivasculitis and vasculitis comprised mainly of IBA1+ cells, variable CD3+ cells, and rare CD20+ cells. In the testis, PCV3-RNA was present within all the pampiniform plexus vessels, tunica albuginea, and rarely in interstitial small vessels. In the pinna, in addition to the perivasculitis and vasculitis, PCV3-RNA was observed within and around the nerve bundles close to the cartilage, often infiltrated by small to moderate numbers of IBA1+ and CD3+ cells.

Conclusions PCV3-associate inflammation in aborted fetuses comprised of IBA1+ macrophages and CD3+ T-cells. As observed in the pampiniform plexus, the medium and large caliber vessels seem severely affected by PCV3 infection**. In the pinna, PCV3-RNA, IBA1+, and CD3+ cells were in the nerve fascicles suggesting the virus association with dorsally rotated ears in these animals.**

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A NOVEL ERYTHROCYTIC IRIDOVIRUS IN A BUTTER FROG

Lina Crespo Bilhalva[1,2], Laura Sander Peres[2], Francisco O. Conrado[1], Andrea Pires dos Santos[3], Eliane Dallegrave[4], Stella de Faria Valle[2]
[1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA, [2]Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3]Purdue University, West Lafayette, IN, USA, [4]Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

Background: More than a third of amphibian species are endangered due to several factors that can be exacerbated by environmental imbalances. Understanding population declines and their infectious diseases is essential for conservation measures. However, there is an overall lack of published date regarding viruses that affect amphibians, which are important pathogens that can lead to outbreaks and, occasionally lethal, clinical disease.

Objectives: This study aimed to describe the morphologic and molecular findings of an erythrocytic virus in a free-living butter frog (*Leptodactylus luctator*).

Methods: An EDTA-anticoagulated blood sample was collected by femoral venipuncture. The amount of blood collected was insufficient for a CBC, but fresh blood smears stained with Wright-Giemsa were evaluated by light microscopy. An aliquot of the sample was submitted for conventional PCR using primers targeting the *Ranavirus* major capsid protein and viral DNA polymerase genes.

Results: Blood smears showed marked anisocytosis and polychromasia, a large number of erythroplastids and occasional trypanosomes. Different intracytoplasmic inclusions were observed in erythrocytes: a crystalloid, pale, polygonal inclusion, and a small, round to oval, slightly eosinophilic inclusion. Leukocyte morphology was unremarkable. Ranavirus DNA was not detected, but the sample was positive using pan-DNA virus primers. Sanger sequencing resulted in a 364 bp consensus sequence with 84%, 70%, and 64% similarity to *Lacerta monticola*, *Rhinella marina*, and *Thamnophis sauritus* , respectively, all of which are erythrocytic viruses.

Conclusion: To date, no erythrocytic virus has been described in the family Leptodactylidae. Phylogenetic analysis should be performed to evaluate the taxonomic position of the virus.

Monday, November 18, 2024
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HISTOPATHOLOGY OF CORALS SHOWING TISSUE LOSS DURING AN UNUSUAL CORAL MORTALITY EVENT IN JAMAICAN NORTH COAST REEFS

Amy Webb[1], Anne Becker[2], Judith Lang[3], Esther Peters[4], Michelle Dennis[1,2]
[1]University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, [2]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [3]Atlantic and Gulf Rapid Reef Assessment, Big Pine Key, FL, USA, [4]George Mason University, Fairfax, VA, USA

Background: An unusual mortality event affecting coral reefs along the northern coast of Jamaica was discovered in 2018. Based on gross lesions and species affected, this mortality event appeared similar to what had been described for stony coral tissue loss disease (SCTLD) in Florida, which is characterized by rapidly progressing tissue loss with high mortality rate affecting >30 species of scleractinian corals.

Objective: The objective of this study was to describe microscopic lesions of diseased corals and determine the extent to which the lesions documented were similar to SCTLD.

Methods: Biopsies were taken from diseased and apparently healthy colonies from 2 sites along the northern coast of Jamaica in spring and summer 2019.

Results: Histologic lesions were nonspecific with the most common being gastrodermal vacuolation, symbiont degeneration/necrosis, symbiont loss, and mucocyte hypertrophy and hyperplasia. The characteristic form of lytic necrosis previously associated with SCTLD was not observed, nor were etiologic agents histologically observed.

Conclusions: It is unclear if this mortality event is the result of a different disease or if it was SCTLD but presenting differential histological changes due to stage of disease when biopsied, host species response to disease, or changes in disease ecology. The lack of bacteria argues against the use of topical antimicrobial therapy commonly implemented in the region. This study highlights the importance of histology in defining coral diseases and the need for selective sampling of active lesions early during a coral disease epidemic.

Monday, November 18, 2024
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HISTOPATHOLOGIC REVIEW OF LESIONS IN 69 FREE-RANGING EGYPTIAN ROUSETTE BATS (ROUSETTUS AEGYPTIACUS) FROM UGANDA

Jessica A. Elbert[1], Brian R. Amman[2], Tara K. Sealy[2], Patrick Atimnedi[3], Jonathan S. Towner[1,2], Elizabeth W. Howerth[1]
[1]University of Georgia College of Veterinary Medicine, Athens, GA, USA, [2]Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA, [3]Uganda Wildlife Authority, Kampala, Uganda

Bats, apart from being among the most populous, widely spread, and diverse mammalian orders globally, serve as effective carriers for numerous viruses of economic and social importance, such as Rabies virus, Hendra virus, and Nipah virus. Egyptian rousette bats (ERBs; *Rousettus aegyptiacus*) are natural reservoirs for Marburg virus and Ravn virus, both viruses belonging to the species *Orthomarburgvirus marburgense* within the family *Filoviridae*. These viruses cause severe, often fatal illness in humans and non-human primates, but little to no clinical disease in ERBs. Understanding the baseline health parameters of a species is crucial for accurately interpreting normal findings and identifying any outliers or abnormalities. To date, a comprehensive histological survey of pathological lesions in wild ERBs has not been conducted, leaving disease prevalence and common comorbidities largely unknown. To address this gap in knowledge, necropsies and histopathological examinations were performed on 69 free-ranging ERBs captured in Uganda in 2008 and 2011. 27/69 (39%) were RT-qPCR-positive in splenic tissue for Marburg virus at the time of capture. Histological changes were found in 25 unique tissues and were categorized as either infectious/inflammatory (406/485, 84%) or non-infectious (79/485, 16%). The most frequent pathological processes were observed in the peripheral nervous system (lymphoplasmacytic perineuritis), vascular system (lymphoplasmacytic perivasculitis), liver (mononuclear or lymphoplasmacytic hepatitis), and haired skin (lymphoplasmacytic interstitial dermatitis). No neoplastic processes were identified. Additionally, more than 60 different endo- and ectoparasites were identified. This data represents the most comprehensive histopathological summary of observed lesions in free-ranging Egyptian rousette bats to date.

Monday, November 18, 2024
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MULTIDISCIPLINARY APPROACH TO PROFICIENCY TESTING IN DETERMINING AN UNKNOWN TOXICANT BY ANIMAL DIAGNOSTIC LABORATORIES

Yang Chen[1], Marissa Petrey[2], Scott Fritz[3], Tim Walsh[3], Sarah Nemser[4], Andriy Tkachenko[4], David Rotstein[5], Megan Miller[4], Angelica Jones[4], Bruce Williams[6], Michael Dark[6], Matthew Kmet[1], Ravinder Reddy[1]
[1]FDA Center for Food Safety and Applied Nutrition, Bedford Park, IL, USA, [2]Institute for Food Safety and Health, Bedford Park, IL, USA, [3]Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS, USA, [4]FDA Center for Medicine Veterinary Laboratory Investigation and Response Network, Laurel, MD, USA, [5]FDA Center For Veterinary Medicine Office of Surveillance and Compliance, Rockville, MD, USA, [6]Davis-Thompson Foundation, Gurnee, IL, USA

Background: The Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) Veterinary Laboratory Investigation and Response Network (Vet-LIRN) is composed of 47 network laboratories in the U.S.A. and Canada that supports FDA's public health mission for food and drug safety. Laboratories test samples for biological and chemical contaminants. To assess laboratory preparedness and competency, Vet-LIRN conducts annual proficiency exercises (PE).

Objective: Evaluate laboratory's ability to identify lead (Pb) toxicosis under conditions mimicking the diagnostic setting of real-world cases requiring an integrative approach among toxicologists, pathologists and chemists.

Methods: Participating laboratories received: (i) a case describing cattle with neurologic signs, (ii) a digital histology slide of brain with morphological abnormalities, which can be associated with several possible causes and (iii) frozen brain and liver specimens for possible chemistry analysis, which had to be determined by participants based on (i) and (ii).

Results: Fourteen participating laboratories submitted the top three differential diagnoses, chemistry testing results and summary of their decision process. All pathologists had similar histopathologic finding of cerebrocortical necrosis. Lead or sulfur toxicosis were top differentials along with thiamine deficiency or sodium toxicosis. Eight laboratories identified lead as the primary differential. Chemistry analysis of brain and/or liver was performed for various elements. Overall, lead toxicosis was correctly identified by 93% (13/14) laboratories.

Conclusions: Most laboratories were able to identify lead toxicosis as the cause of animal illness through an interdisciplinary approach. The PE stressed the importance of the integrative approach to investigations.

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EXPLAINABLE AI FOR SPATIAL BIOLOGY AND HISTOLOGICAL ANALYSIS OF CANINE HEMANGIOSARCOMA

Donghee Lee, Rohith Sai Reddy Kondreddy, Hyunji Jo, Joanne Kim, Heo Heon, Jong Hyuk Kim
University of Florida, Gainesville, FL, USA

Background: Hemangiosarcoma (HSA) is an aggressive vascular cancer prevalent in domestic dogs. This tumor forms malignant endothelium and irregular vascular channels, often containing blood clots. The unique pathognomonic features of HSA may present diagnostic challenges, and the spatial organization of the malignant vasculature remains virtually unknown.

Objective: This study aimed to develop explainable AI models to advance the understanding of the spatial biology of HSA and to facilitate computerized histological analysis.

Methods: We utilized convolutional neural network models, ResNet-50 and AlexNet, to identify malignant tissues in HSAs, with Grad-CAM visualization. Our dataset comprised 4,316 images of hematoxylin and eosin (H&E) stained slides from seventy visceral HSAs and 3,536 images of thirty splenic hematomas, used as benign controls, obtained from client-owned dogs. We then generated whole slide images (WSI) of H&E slides to perform deep learning-based histological analysis and created heatmaps to dissect distinct tissue components.

Results: Our results showed that ResNet-50 and AlexNet models achieved training accuracies of 99.07% and 98.39%, respectively, in classifying HSA from benign images, with AUCs of 0.999 and 0.998. The Grad-CAM results highlighted malignant tissue components utilized in their decision-making process. Moreover, AI-implemented WSI analysis enabled the segmentation of tissue into tumor regions and other components, providing spatial heatmaps.

Conclusions: This study demonstrates that histological images of canine HSA are trainable by explainable AI, offering a powerful digital platform to study the spatial biology. Our ongoing work includes integrating histological images with genomic datasets to build comprehensive models for the molecular phenotyping of HSA.

Monday, November 18, 2024
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IMMUNOHISTOCHEMICAL AND HISTOMORPHOLOGIC CHARACTERIZATION OF CANINE EXTRA-ADRENAL NEUROENDOCRINE NEOPLASMS

Christopher Champion, Andrew Miller, Stephen Parry, Elena Alina Demeter
Cornell University, Ithaca, NY, USA

Background: Canine extra-adrenal neuroendocrine neoplasms (CEANNs) are an enigmatic and heterogeneous group of tumors with poorly defined immunohistochemical and histomorphologic characteristics.

Objective: To characterize the immunohistochemical profile and histomorphologic features of canine extra-adrenal neuroendocrine neoplasms.

Method: Tissue microarrays were assembled using formalin-fixed paraffin-embedded (FFPE) blocks from 82 CEANNs. The histomorphologic characteristics and immunohistochemical profiles were assessed. The following immunohistochemical assays (IHCs) were utilized: chromogranin A, neuron-specific enolase (NSE), microtubule-associated protein 2 (MAP2), pan-cytokeratin, oligodendrocyte transcription factor 2 (OLIG2), protein gene product 9.5 (PGP9.5), vimentin, synaptophysin, neuronal nuclei (NeuN), S100, SRY-related HMG-box 10 (SOX10), glial fibrillary acidic protein (GFAP), insulinoma-associated protein 1 (INSM1), and antigen Kiel 67 (Ki67).

Results & Conclusions: The proportion of cases that immunolabeled with each IHC was as follows: PGP9.5 = 77/82 (93.9%); NSE = 68/82 (82.9%); synaptophysin = 59/82 (72.0%); chromogranin A = 56/82 (69.3%); INSM1 = 37/82 (45.1%); MAP2 = 32/82 (39.0%); vimentin = 24/82 (29.3%); OLIG2 = 7/82 (8.5%); pan-cytokeratin = 5/82 (6.1%); SOX10 = 1/64 (1.6%); S100 = 1/67 (1.5%); NeuN = 1/82 (1.5%); GFAP = 0/63 (0%).

PGP9.5, NSE, synaptophysin, and chromogranin A were positive in over 50% of cases. 81/82 (98.8%) of cases immunolabeled with at least one of these 4 IHCs and thus, these four IHCs are deemed most useful when attempting to substantiate that a neoplasm is of neuroendocrine origin.

Chromogranin A, MAP2, synaptophysin, INSM1, and vimentin immunolabeling varied significantly between anatomic locations. The only histomorphologic feature that varied significantly between anatomic locations was the presence/absence of intracytoplasmic granules.

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HISTOLOGICAL CHANGES IN MESENTERIC GANGLIA VERSUS SURVIVAL OF DOGS WITH DYSAUTONOMIA

Gayle Johnson, Dae Kim, Kei Kuroki, Dennis OBrien, Rosalie Ierardi, Annabelle Burnum-Looney, Renata Mammone, Amanda Smith, Alexis Carpenter
University of Missouri, COLUMBIA, MO, USA

Canine dysautonomia is an idiopathic disease of young dogs that is characterized by widespread loss of autonomic neurons. The density of autonomic neurons in mesenteric ganglia is reduced by four to five-fold compared to unaffected dogs. Retrospective examination of celiac-anterior mesenteric enteric ganglia determined neuronal density by point/point counts of grid intersects landing on neurons. Median intersects for affected dogs averaged 4.8% while the figure for normal dogs was 21.7%. Using 200 neuron counts and calculating the density of basophilic neurons, revealed a wide range of basophilic neuron density between patients surviving 1-17 days after onset of signs, with a relative, but non-significant increase from 18-28 days. The number of surviving dogs was small.

HE-stained sections from 57 postmortems were examined where the duration of illness was documented. Microscopic findings had no exact timeline, possibly due the variation in individual animals. Autonomic ganglia from control dogs, had > 99% basophilic neurons. In affected dogs euthanized during the first 2 days there was mild reduction in neuronal density with continued basophilia in some patients. By day 3, most neurons had chromatolytic changes, or were swollen. Distinct small or large unstained vacuoles occurred in the cytoplasm. Cell density was decreased during this period. After day 18, neurons began to recover and the interstitium was less expanded. In some cases, many of the remaining neurons regained Nissl substance. This suggests that under some circumstances, ganglion cells can survive dysautonomia and regain the pre-disease characteristics.

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CANINE HISTIOCYTIC PROLIFERATIVE DISEASE (HPD): CATEGORISATION USING HISTOPATHOLOGY, DIFFERENTIALLY EXPRESSED GENES, DIGITAL CYTOMETRY AND CLINICAL PROGRESSION REVEALS INDIVIDUAL CASE DISCORDANCE.

Sam Beck[1], Greet Junius[2], Matthew Smalley[3]
[1]Independent Anatomic Pathology Ltd, Bath, United Kingdom, [2]Medvet BV, Antwerp, Belgium, [3]Cardiff University, Cardiff, United Kingdom

Background

Canine histiocytic sarcoma (HS) is a clinically aggressive neoplasm associated with rapidly progressive disease and death. Predisposed breeds include Bernese Mountain dogs (BMD), Rottweilers and Flat coated retrievers. In contrast, cutaneous histiocytoma are benign, localised skin tumours, with spontaneous, immune mediated resolution expected in the majority of cases. Reactive histiocytosis is an enigmatic histiocytic proliferative disease, observed in a similarly predisposed pedigree group. Clinical and pathological categorisation is often challenging in diagnostic practice.

Objective

Explore histopathological categorisation of HPD from a cohort (13) of predisposed breeds comparing RNA seq differentially expressed genes, Cibersort digital cytometry and clinical progression.

Results

The majority of histopathological diagnoses were associated with an expected clinical outcome and close association of their differentially expressed gene profiles. Individual cases displayed unexpected discordance between histopathology, genetic categorisation and clinical behaviour. For example an atypical population of IBA1 positive cells displaying multinucleation, nuclear pleomorphism and multinucleation in a BMD was classified as HS histologically, grouped with other cases of HS under RNA seq principal component analysis but clinically behaved as a cutaneous histiocytoma. There is an apparent shared inflammatory microenvironment described with Cibersort between cutaneous reactive histiocytosis and histiocytoma. Individual cases of histiocytoma display similarity to HS with digital cytometry but behave as benign tumours clinically.

Conclusions

RNA seq and Cibersort are useful tools for exploring histiocytic proliferative disease using archival material. Pilot comparison between the histiocytic proliferative entities highlights expected similarities in grouping for the majority of cases, with interesting exceptions indicating the need for further study.

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A NOVEL DEEP LEARNING ALGORITHM TO DIFFERENTIATE FELINE INFLAMMATORY BOWEL DISEASE AND SMALL INTESTINAL LYMPHOMA

Brian LaMendola[1], Gerald Duhamel[2], Parminder Basran[3], Ryan Taylor[1]
[1]Department of Population Medicine and Diagnostic Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Department of Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [3]Department of Clinical Sciences, Section of Medical Oncology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Routine histological confirmation of feline small intestinal lymphoma from inflammatory bowel disease (IBD) can be challenging based on endoscopic biopsy specimens alone. This is due to the perceived continuum of disease and intra- and inter-observer variability during evaluation. A machine-learning approach may improve diagnostic accuracy while also supporting more effective diagnostic decisions.

Objective: We aimed to develop a deep learning (DL) algorithm to guide diagnostic decisions and increase diagnostic confidence when differentiating feline IBD and small intestinal lymphoma with endoscopic biopsy specimens.

Methods: A retrospective search of the Cornell Animal Health Diagnostic Center Anatomic Pathology archives using strict inclusion criteria yielded 156 cases with a diagnosis of lymphoplasmacytic enteritis (n=68) or small intestinal lymphoma (n=88). To reduce inter-observer variability and to confirm the initial diagnosis, each case was reviewed by two ACVP diplomats. Whole slide images from 122 confirmed cases were created and fed through a Clustering-constrained Attention Multiple Instance Learning (CLAM) deep learning algorithm. This algorithm classified each whole slide image and produced a heat map to identify sub-regions of high diagnostic value that drove the algorithm's decision making. The remainder of the cases were retained for validation of the algorithm through comparison of the results against a panel of two ACVP diplomats.

Results/Conclusion: Preliminary models show promising results with an area under the curve (AUC) of up to 0.875. We believe that further refinement of the algorithm will result in a diagnostic tool with the potential to guide the diagnosis of feline IBD and small intestinal lymphoma.

Tuesday, November 19, 2024
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PREVALENCE, RISK FACTORS, AND PROGNOSIS OF NEOPLASIA IN SUGAR GLIDERS (PETAURUS BREVICEPS)

Judith Kovach-Zukin[1], Michael M. Garner[2], Elise E. B. LaDouceur[1,2]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]Northwest ZooPath, Monroe, WA, USA

Introduction: Sugar gliders are small, arboreal marsupials native to Australia and are increasingly popular pets. This study summarizes neoplasms diagnosed in captive sugar gliders submitted to Northwest ZooPath (NWZP) and includes prognostic and risk factors.

Methods: NWZP archives were searched for neoplasia in sugar gliders. Odds ratios were performed to determine risk factors and prognoses.

Results: 77/251 necropsy and biopsy specimens from sugar gliders had neoplasia (30.7% prevalence) in the integumentary (n=22; soft tissue sarcoma n=5), hepatobiliary (n=21; hepatocellular adenoma n=10, hepatocellular carcinoma n=8), reproductive (n=17; mammary carcinoma n=12),

hematopoietic (n=7; round cell neoplasia [lymphoma]), digestive (n=5), musculoskeletal (n=4), endocrine (n=2), urinary (n=2), and cardiovascular (n=1) systems. Animals with lymphoma were more likely to be <7 yo than ≥7 yo (p=0.0286). Animals with mammary carcinoma were more likely to be female than male (p=0.0079). Animals with hepatomas were more likely to present for necropsy than biopsy (p=0.0268), but it was unlikely for death to be attributed to hepatomas (p=0.0039). In animals that presented for necropsy, mammary carcinomas were more likely to metastasize than other neoplasms (p=0.0017).

Conclusions: Neoplasia is common in sugar gliders. Hepatomas were diagnosed at necropsy, but were not associated with death, reflecting their incidental nature. The high likelihood of metastasis in mammary carcinomas indicates that this is an aggressive neoplasm in sugar gliders. All types of metastatic neoplasms metastasized to the lungs, which had no identified primary neoplasms in this study, suggesting pulmonary masses in sugar gliders should prompt screening for masses in other anatomic locations.

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LETHAL, DISSEMINATED INFECTION WITH HALIOTICIDA NODULIFORMANS IN CAPTIVE ISOPODS (BATHYNOMUS GIGANTEUS AND BATHYNOMUS DOEDERLEINII) IN THE UNITED STATES

Elise LaDouceur[1,2], Thomas Cotrone[1], Michael Murray[3], Ri K. Chang[3]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]Northwest ZooPath, Monroe, WA, USA, [3]Monterey Bay Aquarium, Monterey, CA, USA

Introduction: *Halioticida noduliformans* is an oomycete that infects external tissues of abalone, mantis shrimp, and European lobsters. *H. noduliformans* has variable pathogenicity depending on the host species; it is lethal in abalone, associated with morbidity in mantis shrimp, and causes death of roe in European lobsters.

Methods: Giant deep-sea isopods (*Bathynomus giganteus*) and Japanese isopods (*Bathynomus doederleinii*) were wild caught and housed in an aquarium in California. Animals that died were examined histologically. PCR with panfungal primers was performed in four cases.

Results: Ten isopods (seven *B. doederleinii* and three *B. giganteus*) died or were euthanized over a 6-month period. These isopods had hemocytic inflammation and necrosis centered on hyphae, which were considered the cause of death. Hyphal morphology was typical of oomycetes with both vegetative hyphae and zoosporangia. Inflammation and hyphal infection were in the body wall (including pleopods; n=10), gills (n=7), hepatopancreas (n=4), eyes (n=3), and gut (n=1). PCR amplified *H. noduliformans* (100% sequence identity with GenBank acc # AB285227).

Discussion: This report describes *H. noduliformans* infection in previously unreported hosts, isopods. In isopods, this pathogen infected both external and internal viscera, the latter of which has not been previously reported in any host species. It also demonstrates that this pathogen occurs in a previously unreported geographic location (i.e., the United States). *H. noduliformans* was considered the cause of death in these isopods. Natural hosts and reservoirs for *H. noduliformans* are still unknown, limiting our ability to understand and control this disease.

Tuesday, November 19, 2024
02:45 PM – 03:00 PM

PURPUREOCILLIUM LILACINUM-ASSOCIATED GRANULOMATOUS PNEUMONIA IN VIRGINIA OPOSSUMS (DIDELPHIS VIRGINIANA) FROM LOUISIANA

Naomi Falconnier, Jeongha Lee, Fabio Del Piero, Mark Mitchell, William Holl, Emi Sasaki, Cesar Menk, Luis Tondo, Matias Dorsch, Bianca de Cecco, Ingeborg Langohr, Mariano Carossino
Louisiana State University, Baton Rouge, LA, USA

Background: Verminous pneumonia, spontaneous pulmonary neoplasms, and endogenous lipid pneumonia are well described in Virginia opossums (*Didelphis virginiana*). A series of unrecognized fungal pneumonias in opossums prompted further investigation.

Objective: The goal of this study was to characterize fungal-associated granulomatous pneumonia and other pulmonary lesions in Louisianan opossums.

Methods: A retrospective analysis of 28 necropsied opossums submitted to Louisiana State University Diagnostics (LSUDX) included cases between 2019 to 2024. Lung sections were evaluated with routine and Grocott-Gömöri's methenamine silver stains. Relevant ancillary testing included lung fungal (1/28) and bacterial (2/28) cultures, fungal ITS-2 PCR and sequencing of lung (4/28) and fungal isolate, and fecal exams (17/28).

Results: A distinctive fungal organism was histologically identified in 75±16% (95% confidence interval) of cases (21/28) as intrahistiocytic, 2-4 µm in diameter, argyrophilic ovoid organisms. Moderate to severely affected lungs were grossly consolidated, with characteristic patchy to generalized indistinct <1-2 mm light yellow parenchymal foci, which corresponded to alveolar filling with foamy macrophages and multinucleated giant cells. Fungal culture and PCR with sequencing of a subset of fungal cases were consistent with *Purpureocillium lilacinum* (4/21). Other findings included verminous pneumonia (13/28; 46.4±18.4%), pulmonary neoplasms (7/28; 25±16%), bacterial pneumonia (5/28; 17.8±14.2%), and endogenous lipid pneumonia (2/28; 7.1±9.4%). Therefore, finding *P. lilacinum* at necropsy was as likely as verminous pneumonia.

Conclusions: *Purpureocillium lilacinum* was associated with granulomatous pneumonia in Louisianan Virginia opossums, which was a common pulmonary finding. Despite significant pulmonary changes, respiratory signs were rarely noted in these opossums, even in severely affected lungs.

Diagnostic Pathology Posters

1: PHEOCHROMOCYTOMA WITH WIDESPREAD AND DERMAL METASTASIS IN A 9-YEAR-OLD BLACK RUSSIAN TERRIER

Taylor Alley, Adam Harris, Kelly Hughes
Colorado State University, Fort Collins, CO, USA

A 9-year-old Black Russian Terrier, with a one-year history of a previously diagnosed left pheochromocytoma with caudal vena cava and left renal parenchyma invasion treated with radiation therapy and medical management, presented for a dermal mass. On cytology the mass was consistent with an epithelial neoplasm with a neuroendocrine appearance (loosely cohesive epithelial cells with rosette-like formations). The patient was euthanized two months later due to clinical decline and poor quality of life. On necropsy, the left adrenal was markedly enlarged (3.5x1x2cm) and effaced by a mass which was fully occluding the caudal vena cava, with invasion into the renal veins, left phrenicoabdominal vein, portal vein; neoplastic cells were also in the pulmonary and cutaneous vasculature. Additionally, multiple masses were found in the kidney, liver, lung, and skin. Histology of

the left adrenal, liver, kidney, lung, and the skin masses identified sheets and packets of polygonal to pleomorphic cells with abundant granular eosinophilic cytoplasm and round to ovoid nuclei with coarse chromatin and a single prominent nucleolus. The cells were variably pleomorphic with areas of marked anisocytosis and anisokaryosis and numerous karyomegalic cells with nuclei up to 35um diameter. Neoplastic cells were negative for MUM1, IBA-1, and CD204 and strongly had positive immunoreactivity for synaptophysin and chromogranin A, consistent with a diagnosis of a pheochromocytoma with widespread metastasis and severe vascular invasion. Although pheochromocytomas are known to show vascular invasion, the cellular pleomorphism, extensive distant vascular infiltration and cutaneous manifestation were unusual in this case.

2: MULTIORGAN LIPID STORAGE DISEASE IN A YOUNG ADULT FELINE

Amelia Andersson, Brittany Baughman
Department of Pathobiology and Population Medicine, Mississippi State University College of Veterinary Medicine, Starkville, MS, USA

A 2-year-old neutered male Domestic Shorthair feline presented to Mississippi State University College of Veterinary Medicine for progressive “poor doing” at home. The owners stated the cat was FIP positive and despite receiving treatment (specifics not disclosed) continued to decline. Due to a poor prognosis, humane euthanasia was elected, and the body was submitted for postmortem examination. On gross evaluation, the oral mucous membranes, sclera, and pinna were white to pale tan. Within the thoracic cavity, the lungs were yellow to tan and the pulmonary pleura contained multifocal tan plaque-like lesions. The liver, spleen, and kidneys were moderately to severely enlarged with rounded margins, were tan to yellow and bulged on cut surface. On histopathologic examination, alveolar septae were diffusely thickened and expanded by numerous macrophages containing intracellular lipid and cholesterol clefts. Similar intrahistiocytic lipid and cholesterol aggregates were present in large numbers throughout the hepatic parenchyma, splenic white pulp, renal glomeruli and cortical tubules, lymph nodes, bone marrow, small and large intestines, and within the choroid plexus and neuropil in the brain. The severity and distribution of multiorgan intrahistiocytic lipidosis and cholesterol clefts are consistent with a lysosomal storage disease, most similar to Neimann-Pick disease described in human medicine. Frozen spleen was submitted to NeoGen for CatScan genetic screening which was inconclusive. Information regarding Neimann-Pick disease in veterinary medicine is outdated and poorly described with no known breed, sex, or age predisposition. Genetic testing or electron microscopy are required for definitive diagnosis and there are no current treatments reported.

3: LARYNGEAL OSSEOUS METAPLASIA IN COMPANION ANIMALS

Daniel Felipe Barrantes Murillo[1], Tatiane Terumi Negrão Watanabe[2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]Antech Diagnostics, Mars Petcare Science & Diagnostics, Fountain Valley, CA, USA

Background: Laryngeal ossification is a form of heterotopic ossification widely recognized in humans and seldom reported in companion animals, with selected case reports available in domestic and wild animals.

Objective: To determine the prevalence and demographic trends of laryngeal osseous metaplasia in companion animals.

Methods: Through a retrospective multi-institutional study, we collected laryngeal specimens for canine and feline patients submitted for autopsy. The relevant clinical information, including age, breed, sex, weight, and final diagnosis was recorded in each case. Histological examination assessed the percentual ossification in four standardized anatomical plane sections of the laryngeal cartilages and tracheal rings.

Results: Cricoid and thyroid ossification was reported in 70.9% (88/124) and 57.2% (71/124) of the canine cases respectively and in 11.1% (3/27) and 3.7% (1/27) of the feline cases. Interestingly the cricoid ossification differed significantly among the body weight (*p*-value = <0.001), sex (*p*-value = <0.011), and age (*p*-value = <0.005) with the higher ossification recorded in larger and older males. The thyroid ossification in dogs differed significantly only with the body weight variable (*p*-value <0.001) with higher scoring in large breeds. Through a logistic regression model, body weight was identified as a risk factor for osseous metaplasia in the cricoid and thyroid cartilage in dogs. Primary laryngeal disease was seldom observed in dogs with marked ossification.

Conclusions: Thus, we support laryngeal ossification as a common incidental finding in dogs related to the age, sex, and breed of the animal. Laryngeal osseous metaplasia is a rare finding in cats.

4: CYTOTOXIC DERMATITIS WITH CORNEAL INVOLVEMENT IN A DOG

Katherine Bauer, Brittany Rasche
Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS, USA

Cytotoxic dermatitis is characterized by keratinocyte death mediated by cytotoxic T lymphocytes and is further subdivided into either interface or panepidermal cytotoxic dermatitis based on the location of keratinocyte death in the basal cell layer or throughout all layers of the epidermis, respectively. Here, we report a case of severe panepidermal cytotoxic dermatitis in a 6-year-old, spayed female, mixed breed (terrier cross) dog, considered most consistent with erythema multiforme (EM). This case is unusual in that there was associated ocular involvement, with bilateral corneal ulcers developing concurrently with erosive to ulcerative cutaneous lesions over an approximately 8-month long history of intermittent flares that were partially responsive to antibiotics and immunosuppressive therapy. Due to recurrence and severity of cutaneous lesions as well as development of oral lesions resulting in dysphagia and anorexia, humane euthanasia was elected, and the animal was submitted for necropsy. Gross examination revealed severe erosive to ulcerative dermatitis affecting both pinnae, oral mucocutaneous junctions, and paw pads. On histologic examination, there was evidence of ulcerative dermatitis and bilateral keratitis with multi-level apoptotic keratinocytes within the epidermis and corneal epithelium, respectively. Although, to our knowledge, ocular involvement in EM has not been reported in veterinary species, in humans, both EM and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) may be associated with ophthalmic manifestations that can result in loss of visual acuity. Monitoring for ophthalmic lesions in canine cases of suspected EM or SJS/TEN may be pertinent as this may be a rare but important manifestation of these diseases.

5: CANINE ADENOVIRUS-1 IN A SIX-WEEK-OLD MIXED BREED PUPPY

Julie Bedwani, Jillian Athey, Miranda Vieson
Veterinary Diagnostic Lab, University of Illinois Urbana Champaign, Urbana, IL, USA

A 6-week-old, female, intact mixed breed puppy was noted to become acutely neurologic exhibiting anti-social behavior and increased vocalization. The puppy lost interest in nursing and died the same day. One week prior a littermate had similar signs and was euthanized. These puppies were a litter of seven born to a dam with an unknown history. Potential clinical differential diagnoses include canine distemper and bacterial or protozoal meningitis.

At necropsy, the puppy was noted to have hemorrhagic contents in the stomach and orad small intestine, moderate fibrinous peritonitis with peritoneal effusion, and mild pleural effusion. Histopathology revealed a severe multifocal necrotizing hepatitis with intranuclear hepatic and rarely endothelial viral inclusion bodies, multifocal acute microhemorrhages of the brain with intranuclear endothelial viral inclusion bodies, myeloid hyperplasia with erythroid hypoplasia, and splenic extramedullary hematopoiesis. Ancillary testing for Campylobacter, canine herpesvirus-1, and canine parvovirus-2 were negative. Canine adenovirus-1 was positive.

Cause of death was attributed to canine adenovirus-1 (CAdV-1), also known as canine infectious hepatitis. Spread by the oronasal route, the prevalence of this virus has significantly reduced in thanks to vaccination campaigns. CAdV-1 has tropism for endothelium, mesothelium, and hepatic parenchyma and damage of these cells leads to features of edema, serosal hemorrhage, and hepatic necrosis. Large, solid eosinophilic to basophilic intranuclear inclusion bodies can be found in endothelium or hepatic parenchyma. Central nervous system manifestations, such as this case, are rarely reported in the literature.

6: PROXIMAL HUMERUS CLEAR CELL CHONDROSARCOMA IN A 6-YEAR-OLD GERMAN SHEPHERD DOG

Hasti Azarabad[1], Nadi Maazi[2], Movahed Khoei[3], Kimia Berenji[1], Yasin Dibavand[1]
[1]Department of Pathology, Hamidreza Fattahian pet hospital, Tehran, Islamic Republic of Iran, [2]Department of internal medicine, Eram pet hospital, Tehran, Islamic Republic of Iran, [3]Department of diagnostic imaging, Hamidreza Fattahian pet hospital, Tehran, Islamic Republic of Iran

Destructive bone lesions in canines are mostly of neoplastic origin, with nearly all primary bone tumors being malignant. In a clinicopathologic study involving 97 dogs with chondrosarcoma, the average age at diagnosis was 8.7 years and Golden Retrievers were at a higher risk compared to other breeds, and 61% of the tumors were located in flat bones. Clear cell chondrosarcoma is a highly uncommon, low-grade malignant neoplasm of bone, accounting for 1.6%–5.4% of all chondrosarcomas. We report the case of a 6-year-old female German Shepherd dog who presented with pain, dehydration and localized swelling. Upon examination, a palpable mass was discovered near Metaphysis of the proximal humerus. Radiologic examination was performed to confirm the diagnosis. On radiographs, well-defined osteolytic lesion with mineralization and sclerotic borders were seen. In histopathologic examination, with hematoxylin and eosin staining, moderately differentiated neoplastic chondrocytes with cytoplasmic vacuoles, central nuclei and little intervening matrix were seen. There were small trabeculae of reactive bone mixed with numerous osteoclast-type giant cells. Based on the results, surgeons decided to treat the neoplastic area by radical amputation joined with scapulectomy. In this case the patient adapted to the condition, supporting her weight and maintaining normal habits and there were no signs of metastasis but a longer time of surveillance is advocated. There are very few reports of chondrosarcoma and its subtypes in veterinary literature and it is very important to highlight the diagnostic features of this rare tumor to achieve the best treatment.

7: SECONDARY OSTEOMA CUTIS IN A MIX SPITZ TERRIER DOG

Hasti Azarabad[1], Ehsan Khaksar[2], Kimia Berenji[1], Movahed Khoei[3]
[1]Department of Pathology, Hamidreza Fattahian pet hospital, Tehran, Islamic Republic of Iran, [2]Department of clinical science, Azad university, Garmsar, Islamic Republic of Iran, [3]Department of diagnostic imaging, Hamidreza Fattahian pet hospital, Tehran, Islamic Republic of Iran

Osteoma cutis is defined as bone formation in dermal or subcutaneous tissues and is usually classified as primary or secondary. Primary osteoma cutis occurs in normal skin without any evidence of underlying lesions, while secondary osteoma cutis occurs in damaged skin. In veterinary medicine, osteoma cutis is very rare. Primary osteoma cutis accounts for 15% of all osteoma cutis cases and is associated with hereditary diseases, and secondary osteoma cutis, which accounts for the majority of osteoma cutis cases, is associated with inflammation, trauma, or tumors in most cases. The underlying mechanisms of osteoma cutis are unclear and may be caused by a developmental error, which osteoblasts that are differentiated from primary mesenchymal cells migrate to an inappropriate location. Osteoma cutis is rare in dogs and most reported cases are secondary and associated with calcinosis cutis due to hyperadrenocorticism. A 9-year-old female mix spitz terrier with a history of Cushing disease was referred to the veterinary clinic and in hematology tests the remarkable thing was the increase in serum ALP and total calcium levels. The lesions observed by radiological imaging

were removed by surgical incision and referred for histopathological examination. Microscopic examination with hematoxylin and eosin (H&E) revealed a large amount of bone matrix with many thin trabeculae in dermal and subcutis. In some areas there were cystic changes of apocrine glands. Patient died because of cushing disease after 3 months. The purpose of this report is to point out the microscopic findings for the diagnosis of this rare condition.

8: TAENIA CRASSICEPS ASSOCIATED WITH NEURAL CYSTICERCOSIS IN A DOMESTIC DOG IN THE UNITED STATES

Bayla Bessemer[1], Croix Griffin[2], Antoinette Marsh[3], Megan E Schreeg[1], Ronaldo da Costa[2], Patrick Roynard[2], Michael Oglesbee[1], Christopher Premanandan[1]
[1]The Ohio State University Department of Veterinary Biosciences, Columbus, OH, USA, [2]The Ohio State University Department of Veterinary Clinical Sciences, Columbus, OH, USA, [3]The Ohio State University Department of Veterinary Preventative Medicine, Columbus, OH, USA

A 2-year-old female spayed mixed breed dog was presented with a three-month history of seizures. Antibody and molecular based testing on CSF for etiological agents included Neospora, *Coccidioidomyces immitis*, canine distemper virus, WNV, CDV, *Borrelia* spp., and *Echinococcus* and were all negative. Coalescing intra-axial complex cystic lesions in the right cerebral hemisphere identified on MRI were suggestive of hydatid cysts. The dog was euthanized after three months of treatment due to worsening signs and was submitted for postmortem examination.

Coalescing 0.5-3 cm cavitations effaced approximately 20% of the left and 40% of the right cerebral hemispheres and contained numerous 3-5 mm long ovoid to elongate soft white to clear metacestodes. Similar structures extended into the subarachnoid space. Histology was consistent with invaginated cysticerci present in bladder compartments. Cysticerci each had a scolex, convoluted invaginated spiral canal, and spinous tegument with numerous calcareous corpuscles. Within many of the cysticerci, there were visible armed rostellum with refractile hooks and muscular suckers. Light microscopic evaluation of whole cysticerci preserved in ethanol revealed rostellar hooks with blade to guard length and handle to guard length measurements comparable with *Taenia crassiceps*. Sequencing of PCR amplicons confirmed 100% sequence identity to *T. crassiceps.*

To date, this is the first known report of canine neural cysticercosis attributed to T. *crassiceps.* T. *crassiceps* has been reported to cause neural cysticercosis in two humans and one cat naturally and is gaining attention for its zoonotic potential.

9: RHABDOMYOSARCOMA IN A PREHENSILE-TAILED PORCUPINE (COENDOU PREHENSILIS)

Bayla Q. Bessemer[1], Megan E. Schreeg[1], Layne Staab[2], Jeff R. Hayes[1]
[1]The Ohio State University College of Veterinary Medicine department of Veterinary Biosciences, Columbus, OH, USA, [2]Boonshoft Museum of Discovery, Discovery Zoo, Dayton, OH, USA

A captive 4-yr-old female prehensile-tailed porcupine (Coendou prehensilis) had quill loss caudal to the right ear associated with a ~1-2 cm soft tissue mass effect. The mass was surgically debulked and described as a poorly differentiated neoplasm on histology. Ten days after surgery, the porcupine became anorectic and severely ataxic with a moderate left sided head tilt, and was euthanized and submitted for a postmortem examination. Deep to the surgical site was a 7x3x0.5cm amorphous, tan, soft, irregularly shaped mass that encircled the right ear canal and invaded skeletal muscle and the tympanic bulla. Histopathology revealed a hypercellular, infiltrative and destructive, unencapsulated neoplasm comprised of interlacing bundles, streams, and herringbone arrangements of spindle cells supported by a scant fine fibrovascular stroma. Cells were oval with indistinct cell margins, scant to moderate granular eosinophilic cytoplasm, and coarsely stippled chromatin with 1-3 nucleoli.

Anisocytosis and anisokaryosis were moderate, with high mitotic activity and occasional multinucleated giant cells. Neoplastic cells invaded through adjacent myofibers and bone of the tympanic bullae immediately adjacent to nerves, consistent with antemortem neurologic signs. Neoplastic cells exhibited cytoplasmic immunolabeling for vimentin, smooth muscle actin (SMA), and desmin, and were immunonegative for S-100 and CD-31. The mass was diagnosed as a rhabdomyosarcoma. To our knowledge, this is the first diagnosis of a rhabdomyosarcoma in a prehensile-tailed porcupine. This case report expands the current knowledge of neoplasia in this species while providing an opportunity to validate immunohistochemistry in this species.

10: PARVOVIRAL MYOCARDITIS IN A 28 DAY OLD MIXED BREED PUPPY WITH DUODENAL AND JEJUNAL CRYPT ABSCESSES

Emily Blackwell[1], Kristin Jacob[2], John Trupkiewicz[1]
[1]Johns Hopkins University, Baltimore, MD, USA, [2]CVCA (Cardiac Care for Pets), Columbia, MD, USA

Despite high rates of vaccination, canine parvovirus (CPV) remains an important cause of neonatal myocarditis. This frequently fatal manifestation of CPV infection occurs when puppies are infected within the first 15 days of life and are unprotected by maternal antibodies. In contrast, enteritis typically follows infection at 8 weeks or older. A 28-day-old mixed-breed male puppy presented for post-mortem examination following sudden death with a history of CPV exposure. The dam developed diarrhea and tested positive for CPV two days postnatal and was subsequently separated from the litter. At nine days old, troponin I levels were elevated at 3.6ng/mL (<0.15ng/mL) in this puppy, with lower elevation noted in 2/4 littermates. Echocardiogram performed at 27 days (one day prior to death) revealed moderate left ventricular dilation and decrease in contractility with one extra systole. Echocardiogram was normal for all other littermates. Necropsy revealed pronounced myocardial pallor consistent with myocardial necrosis, mucosal membrane pallor, pleural effusion (4.5mL), and mild jejunal parasitism. Histology revealed severe nonsuppurative myocarditis with intranuclear viral inclusions, and immunohistochemistry confirmed the presence of CPV antigen. Interestingly, multifocal crypt abscesses were identified in the duodenum and proximal jejunum, and immunohistochemistry failed to detect local CPV. Few intraluminal nematodes identified in the most aboral portion of jejunum are likely unrelated. The cause of intestinal lesions is undetermined. All other littermates survived, and repeat echocardiogram at 8 weeks revealed hyperechoic myocardial foci which may indicate fibrosis. This case report describes a classic disease entity supported by clinical data that is frequently unavailable.

11: MYCOBACTERIUM CELATUM MULTISYSTEMIC GRANULOMATOUS DISEASE IN A DOMESTIC FERRET (MUSTELA PUTORIUS FURO) IN TUCSON, AZ

Jennifer Buczek[1], Alexandra Brower[2], Tayna Padilha Basqueroto Antunes[3], Linda Doran[4], Rinosh Mani[3]
[1]Colorado State University, Fort Collins, CO, USA, [2]Midwestern University, Glendale, AZ, USA, [3]Michigan State University, Lansing, MI, USA, [4]Freelance Medical Journalist, Los Angeles, CA, USA

A 5-year-old female spayed domestic ferret in Tucson, AZ had a 4-month history of intermittent hyperthermia, lethargy, and monocytosis with peripheral lymphadenopathy, splenomegaly, and suspected lymphoma. Testing for Aleutian mink disease, adrenal disease, and insulinoma were negative or non-confirmatory. Treatments with prednisolone, cyclophosphamide, and L-asparaginase were variably successful. Euthanasia was elected due to worsening condition. On postmortem examination, there were numerous 1.0 -3.0 mm granulomas within the lungs, kidneys, spleen, and right adrenal gland. On histologic examination, there was multisystemic granulomatous inflammation affecting the lungs, kidneys, spleen, lymph nodes, pancreas, liver, and several blood vessels with small numbers of acid-fast bacillus bacteria in the cytosol of macrophages. Bacteria were elongate, slender, and occasionally branching. Other findings included patchy alopecia, islet cell tumors (2), adrenocortical adenoma, generalized muscle atrophy, endogenous lipid pneumonia, mild

cardiomyopathy, and eosinophilic-lymphoplasmacytic enteritis. Standard bacterial culture and immunohistochemistry for ferret systemic coronavirus disease (FSCD) were both negative. Mycobacterial spp. specific PCR on frozen kidney tissue was negative (16s rDNA) at one diagnostic facility and positive (hsp65) at another with 100% sequence homology to *Mycobacterium celatum.* To our knowledge, this is the first reported case of *M. celatum* in any animal from North America. There are two major conclusions from this case study, 1) *M. celatum* is a differential for multisystemic granulomatous disease causing clinical decline within domestic ferrets in the United States, and 2) certain Mycobacterial spp. present a diagnostic challenge given their unusual morphology and propensity for false negatives on standard culture and various molecular assays.

12: FATAL SODIUM BICARBONATE (BAKING SODA) INGESTION IN A LAMB WITH CONGENITAL NEOSPOROSIS

Anne Burnum, Luis Rivero Acevedo, Elimar Ruiz Cortes, Pamela Adkins
University of Missouri College of Veterinary Medicine, Columbia, MO, USA

A 3-month-old, 11.8 kg, female Katahdin lamb was presented to the MU Food Animal Hospital (FAH) for lethargy, diarrhea, and distended abdomen. Previously, the lamb had been diagnosed with congenital cerebellar disease, having been non-ambulatory with motor present in all 4 limbs, intention tremors, and hypermetria since birth. The night before presentation, the lamb was given 2 oz of a solution of baking soda diluted 1:3 with water for presumptive bloat. Upon presentation, the lamb was dull and laterally recumbent with tachypnea, tachycardia, and mild dehydration (5-7%). Rumen contractions were absent and her extremities were cold. On neurologic examination, she was depressed and mentally inappropriate with non-ambulatory tetraparesis and tremors. Blood chemistry revealed marked hypernatremia (198 mEq/L) and elevated bicarbonate (43 mEq/L). The lamb was started on 185 mEq/L NaCL at a rate of 3 mL/kg/hr to gradually decrease the sodium concentration, and received flunixin meglumine, thiamine, dexamethasone, and ampicillin. However, she developed seizure activity accompanied by hyperthermia (107.4 F) that was poorly responsive to active cooling and IV midazolam, and ultimately died. Histologic examination of the brain revealed laminar neuronal necrosis as well as a chronic non-suppurative meningoencephalitis with multiple tissue cysts containing bradyzoites. *Neospora caninum* qPCR was positive. Additionally, the brain tissue contained 2255 ppm of sodium, consistent with salt toxicosis. Oral administration of baking soda is touted as a readily available over-the-counter remedy for bloat due to rumen acidosis. However, it is important to recognize the danger that sodium bicarbonate overdose poses to livestock and pets.

13: EPIDEMIOLOGY AND PATHOLOGY OF YERSINIOSIS-INDUCED ABORTION IN SMALL RUMINANTS: A 22-YEAR CASE SERIES STUDY AT THE CALIFORNIA ANIMAL HEALTH AND FOOD SAFETY LABORATORY SYSTEM: 2002-2023

Seunghee Cho[1], Asli Mete[2], Isaiah Cueva[2], Melissa Macías-Rioseco[3], Heather Fritz[2], Nicolas Streitenberger[2], Omar Gonzales-Viera[2]
[1]Department of Comparative Pathobiology/ADDL, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA, [2]California Animal Health and Food Safety Laboratory system (CAHFS), Davis Laboratory, University of California, Davis, Davis, CA, USA, [3]California Animal Health and Food Safety Laboratory system (CAHFS), Tulare Laboratory, University of California, Davis, Tulare, CA, USA

Background

Abortion in small ruminants poses a significant economic threat and might have zoonotic causes. While the association between yersiniosis and reproductive complications is known, systematic studies and case series on abortion in sheep and goats are scarce.

Objective

This study aims to characterize the epidemiological and pathological features of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*-induced abortions in small ruminants, contributing to the understanding of this zoonotic disease in California.

Methods

A 22-year retrospective study was conducted to examine microbiological and pathological findings in submissions for abortion diagnostics, as well as the geographic and seasonal distribution of disease.

Results

Yersiniosis-induced abortion was diagnosed in 22 goats and 12 sheep, with all abortions occurring in the 3rd trimester. Samples from lung, liver, placenta, and abomasal contents were submitted for aerobic culture, and abomasal contents showed the highest recovery of *Yersinia* spp. Microscopically, there was severe necrotizing and suppurative inflammation in the lung, liver, spleen, kidney, and, when present, the placenta. There was evidence of hepatic copper, zinc, and selenium deficiency in nine, two, and three cases, respectively. Geographically, cases were concentrated in Northern and Central California, with a seasonal pattern favoring winter and spring occurrences.

Conclusions

In conclusion, this 22-year retrospective study at the California Animal Health and Food Safety Laboratory System significantly contributes to the understanding of the epidemiological and pathological features of *Yersinia spp.*-induced abortions in small ruminants.

14: PITUITARY MACROADENOMA WITH BILATERAL ADRENAL CORTICAL HYPERPLASIA IN A GUINEA PIG

Michael Clayton[1], Nicolle Lofgren[2], Sophie Dennison[3]
[1]Utah State University, Logan, UT, USA, [2]Mountain West Veterinary Specialists, Layton, UT, USA, [3]TeleVet Imaging Solutions, PLLC, Baltimore, MD, USA

In January of 2024, a three-year-old, female guinea pig was presented to the exotic animal service at an emergency and specialty veterinary hospital in Northern Utah. Presenting complaints included a one-month history of progressive ataxia, hyporexia, and lethargy. The guinea pig was obtunded, with severe ptyalism, bilateral otic discharge, and wheezing auscultated over the right lung field. A complete blood count, serum biochemistry panel and serology were performed, and the animal was hospitalized. Complete blood count showed a stress leukogram and an increased packed cell volume (50.4%). Serum biochemistry was within normal limits, while symmetric dimethylarginine (SDMA) was elevated at 16 ug/dL (<12 ug/dL) suggesting possible renal disease. Whole body CT detected peribronchial consolidation, gas distention in the gastrointestinal tract, and a pituitary mass. Serum antibody titers were positive for Encephalitozoon cuniculi. The guinea pig did not improve and was euthanized the following day, then submitted to the Utah Veterinary Diagnostic Laboratory for necropsy. Necropsy findings included a 15x8x5 mm pituitary mass with dorsal compression of the adjacent hypothalamus, and bilateral symmetrical adrenal gland enlargement. The pituitary mass was an expansile epithelial neoplasm most consistent with a pituitary gland adenoma. The adrenal cortices were bilaterally severely thickened. Cystic rete ovaries, focal interstitial pneumonia, portal hepatitis, tubulointerstitial nephritis, and serous atrophy of fat were also observed. Neurologic signs were attributed to central nervous system compression. To the authors knowledge, this is the first report of a pituitary gland macroadenoma with bilateral adrenal cortical hyperplasia in a guinea pig.

15: RENAL AMYLOIDOSIS IN CAPTIVE GAZELLES AND ASSOCIATED LESIONS

Allysa Cole[1], Emily King[1,2], Randall Junge[2], Priya Bapodra-Villaverde[2], Emily Vincent[1,2], Jessica Heinz[3], Megan Schreeg[1]
[1]The Ohio State University College of Veterinary Medicine, Columbus, OH, USA, [2]Columbus Zoo and Aquarium, Columbus, OH, USA, [3]Cincinnati Zoo and Botanical Garden, Cincinnati, OH, USA

Renal amyloidosis has been previously reported in multiple species of gazelle. While there has been some association seen with concurrent inflammatory diseases, additional risk factors and infectious agents have yet to be identified. We therefore performed a retrospective evaluation of renal amyloidosis in gazelles submitted to The Ohio State University College of Veterinary Medicine. There were 14 gazelles submitted between January 2016 and May 2024 that had kidney tissue available for evaluation, which included Dama gazelles (n=4), Rhim gazelles (n=4), a Thompson's gazelle, a Pronghorn gazelle, and four gazelle of unspecified species. Of these gazelles, 8/14 (57%) had renal lesions with 5/14 (36%) having amyloid present within the kidneys which was observed within the medullary interstitium (3/5, 60%) and glomerulus (3/5, 60%). The average age of gazelles with renal amyloidosis was 9 years, while those with no amyloid averaged 3.5 years. Of gazelles with renal amyloidosis, 3/5 (60%) cases had confirmed bacterial infection with associated chronic inflammation in other organs. Those included bronchopneumonia associated with *Actinomyces hyovaginalis*; splenic granulomas associated with *Escherischia coli* and *Listeria monocytogenes*; and dermatitis, panniculitis, and osteoarthritis associated with *Propionibacterium australiense*. Additionally, 3/5 cases with renal amyloidosis, including the case that cultured *E. coli* and *Listeria*, showed amyloid deposition with other organs including the liver (n=2) and spleen (n=2). Future investigation and additional renal histochemical stains are pending. The cases presented herein identify novel infectious agents associated with renal amyloidosis and further explores concurrent lesions.

16: COMPARATIVE PATHOLOGY OF CHOLANGIOCARCINOMA ACROSS SPECIES: INSIGHTS FROM VETERINARY AND HUMAN MEDICINE

Katti Crakes[1], Hannah Stephen[1,2], Amanda Maxwell[1], Kiyoko Oshima[1], John Trupkiewicz[1], Cory Brayton[1]
[1]Johns Hopkins University, School of Medicine, Baltimore, MD, USA, [2]University of Georgia, College of Veterinary Medicine, Athens, GA, USA

Cholangiocarcinoma (CCA) is an aggressive hepatobiliary neoplasm that affects a wide range of species, including humans, dogs, cats, bears, ruminants, birds, and reptiles. Despite being the second most common primary liver tumor in humans, veterinary literature on CCA remains limited to sporadic case reports primarily in companion animals. This study aims to classify gross and histologic subtypes of CCA in veterinary species, drawing parallels with human medicine.

We examined 18 cases of CCA in companion animals, laboratory animals, and zoo/wildlife (mammals, n=9; reptiles, n=6; avian, n=3) submitted to Johns Hopkins Comparative Pathology from 1986-2024. Each case was reviewed for gross and histologic classification based on location (intrahepatic or extrahepatic), gross subtype (mass-forming, perihilar, or intraductal), neoplastic origin (small or large duct), and differentiation (well, moderate, or poor). The majority of cases were intrahepatic and mass-forming (16/18), with neoplastic origins in both large (10/18) and small ducts (8/18). Differentiation varied, with well (9/18), moderate (7/18) and poorly (2/18) differentiated tumors. Metastatic spread was observed in 5/18 cases, involving the spleen, intestines, lung, heart, pancreas, kidney, and mesentery.

Immunohistochemical staining identified CK7 and 19 are identified as reliable biliary markers, whereas CK20 yielded equivocal results. Additionally, six cases initially resembling CCA were reclassified as benign biliary neoplasms, neuroendocrine tumors, polycystic liver disease, and metastatic adenocarcinoma. Finally, we summarize the diagnostic criteria and nomenclature between

human (WHO) and veterinary (WSAVA, INHAND) guidelines, underscoring the need for uniformity in a comparative approach to advance our understanding of translational models and clinical treatment of CCAs.

17: MALIGNANT CHEMODECTOMA IN A LABRADOR RETRIEVER

Rochelle Day[1,2], Brittany Rasche[1,2]
[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS, USA, [2]Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS, USA

A 12-year-old spayed female Labrador retriever was clinically diagnosed with an osteosarcoma and pathologic fracture of the right proximal humerus with suspected pulmonary metastasis and a heart base mass.

Gross postmortem examination revealed multiple nodules along the right heart base and extending into the wall of the main pulmonary artery. Expanding the medulla of the proximolateral aspect of the right humerus was a poorly demarcated mass, consistent with the suspected osteosarcoma. A similar mass was present along the right fifth rib. There were numerous small nodules scattered throughout the lungs.

Histologic examination of the main pulmonary artery/heart base masses and the right proximal humerus and right fifth rib masses as well as the pulmonary nodules revealed similar multilobulated, non-encapsulated, invasive neoplastic aggregates consisting of moderately to densely cellular polygonal to round cells arranged in packets and broad sheets supported by fine fibrovascular stroma. These neoplastic cells exhibited strong cytoplasmic immunolabeling for chromogranin A, consistent with a neuroendocrine neoplasm. Given the presence of masses at the heart base, aortic body carcinoma (malignant chemodectoma) with pulmonary and bone metastases was considered the most likely diagnosis in this case. No histologic evidence of osteosarcoma was found.

Chemodectomas are neuroendocrine tumors arising from the chemoreceptor cells found in the aortic and carotid bodies. Although vascular invasion is noted with aortic body carcinomas, metastasis to the lungs, liver, or bones in dogs is rare. Thus, this represents an unusual case of malignant chemodectoma with pulmonary and bone metastases, which were clinically mistaken for metastatic osteosarcoma.

18: H5N1 CLADE 2.3.4.4B VIRUS INFECTION AMONG GOAT KIDS

Ariel De Aguiar[1], George Kaslow[1], Erica Wietgrefe[1], Mackenzie Reberg[2], Mia Torchetti[3], Jennifer Johnson[3], Kimberly Lehman[3], Rebecca Cox[3], Erik Olson[1]
[1]University of Minnesota, Saint Paul, MN, USA, [2]USDA - APHIS, Saint Paul, MN, USA, [3]USDA-APHIS-VS-D&B-NVSL-DBPL, Ames, IA, USA

Background: The highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b has led to outbreaks among U.S. commercial poultry and backyard flocks since 2022, with documented spillover to various mammal species. In March 2024, several newly kidded farmed goats developed acute neurological signs prior to death. The goat herd shared the pasture and water source with a flock of chickens and ducks, which had been recently depopulated due to HPAI.

Methods and Results: A goat kid from this herd was necropsied with no significant macroscopic abnormalities. Histologic lesions involved cerebrocortical neuroparenchymal vacuolation, gliosis, neuronal degeneration/necrosis, edema, and neutrophilic and lymphoplasmacytic meningoencephalitis. The heart exhibited mild cardiomyocyte necrosis with pleocellular myocarditis. Intralesional influenza A antigen was detected immunohistochemically in the brain and heart. Frozen tissues were tested by matrix and H5 clade 2.3.4.4b-target PCR assays with detections in multiple

organs. Whole genome sequencing and phylogenetic analysis revealed the virus as HPAI H5N1 clade 2.3.4.4b genotype B3.6 with high identity to the virus from the recently depopulated ducks and chickens. Genotype B3.6 replaced genotype B3.2 as the predominant genotype circulating in wild birds during the fall of 2023. Subsequent PCR testing of nine additional mortalities identified four kids with the HPAI virus (as well as concurrent *Coxiella burnetti*); adult nannies tested negative.

Conclusions: This is the first reported natural infection of a ruminant by HPAI H5N1 in North America. The farm's history of HPAI in poultry and the shared environment between the birds and goats likely contributed to cross-species transmission.

19: HEREDITARY ATAXIA SYNDROME IN A DOG

Bianca de Cecco[1,2], Jocelyn Lee[1], Kylie Landry[1], Patty Lathan[1], Jeongha Lee[1,2]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]LSU-LADDL, Baton Rouge, LA, USA

A 1-year and 11-month-old male castrated Collie dog presented with worsening signs of hindlimb weakness and loss of appetite. Owners reported that the dog's littermates had similar neurologic signs. At examination, progressive para/tetraparesis, limited vision, and pale mucous membranes were observed. A clinical diagnosis of severe non-regenerative anemia was made, and due to poor prognosis, the dog was euthanized and submitted for postmortem examination. Grossly, there was severe pallor of the entire body. Microscopically, the lesions were observed in the central nervous system and concentrated in the cerebellum and spinocerebellar tracts and included extensive loss of granular cell layer and Purkinje cells in the cerebellum, and areas of gliosis and spongiosis in the cerebellar, cerebral, spinal cord white matter with prominent reactive astrocytes. Ocular lesions were bilateral and included retinal dysplasia and optic nerve degeneration. Bone marrow examination was supportive of precursor-targeted immune-mediated anemia. The association of progressive para/tetraparesis with spinocerebellar tract degeneration is highly suggestive of an inherited/genetic degenerative disorder similarly described in humans and categorized as spinocerebellar ataxia which falls under the umbrella term "hereditary ataxia". Hereditary ataxias are a heterogeneous group of neurodegenerative diseases characterized clinically by cerebellar or spinocerebellar dysfunction with ataxia as the main clinical sign. These diseases appear to be caused by genetic mutations/defects interfering with basic cellular functions such as autophagy and degradation, cation trafficking, or transport. In our case, the observed lesions in the central nervous system, and ocular structures classify this hereditary ataxia as multifocal degeneration with predominant spinocerebellar component.

20: NON-CHYLOUS LYMPHORRHAGIC PLEURAL EFFUSION AND POLYCYSTIC LIVER DISEASE IN A DOG

Bianca de Cecco[1,2], Jocelyn Lee[1], Andrea Johnston[1], Emi Sasaki[1,2]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]LSU-LADDL, Baton Rouge, LA, USA

An 8-year-old female spayed Maltese dog presented for inappetence, non-productive cough, and increased respiratory rate. Diagnostic imaging identified bilateral pleural effusion, an enlarged liver with multiple cystic structures, and a cystic soft tissue structure adjacent to the pancreas. Analysis of the pleural fluid was consistent with non-chylous lymphorrhage with acute hemorrhage. Despite placement of a pleural-port and octreotide therapy, the owner elected humane euthanasia after just 10 days of therapy. A postmortem examination was performed. On gross examination, the thoracic cavity contained approximately 250 ml of opaque fluid and atelectatic lungs. Hepatomegaly with marked distention of the hepatic parenchyma by numerous cysts was also observed. The kidneys and pancreas were normal. Histologically, liver cysts were lined with flat to plumped cuboidal CK7 positive epithelium, consistent with biliary epithelium. No significant lesions were observed in the pleura or diaphragm. This case was diagnosed as polycystic liver disease and subacute pleural effusion. Polycystic liver disease without associated polycystic kidney disease is rare in dogs and

uncommon in humans. It is characterized by isolated cysts disrupting the hepatic parenchyma. Mechanistically, pleural lymphorrhagic effusion develops secondary to altered permeability of local capillaries and lymphatic vessels on the diaphragmatic pleural surface. Exudative pleural effusions are rare complications of polycystic liver disease. Only 3 human cases have been reported, all of which responded to surgical cyst decompression.

21: CD3+ LYMPHOPROLIFERATIVE NEOPLASIA AND SECONDARY MYOCARDITIS IN A GREEN TREE PYTHON (MORELIA VIRIDIS)

Michelle Degnin, Allison Vilander
Colorado State University, Department of Microbiology, Immunology, and Pathology, Fort Collins, CO, USA

Background: A 7-year-old intact female green tree python (*Morelia viridis*) presented for necropsy following sudden death after failing to gain weight for 3 months.

Methods: A post-mortem exam was performed, and representative samples were taken of major organs and fixed in 10% neutral buffered formalin. Fresh tissue of lung, liver, kidney, spleen, and pericardium were collected using sterile technique. Representative samples of all major organs were stained with hematoxylin and eosin. Immunohistochemistry was performed using CD3 (Leica, LN10), CD20 (epredia/Fisher, RB-9013-P), and PAX5 (Leica, 1EW) on kidney, liver, and lung. Bacterial culture for anaerobes and aerobes were performed on fresh pericardium.

Results: The snake was in good body condition with a markedly thickened pericardium that was adhered to the epicardium. The myocardium was mottled dark red to purple. No other significant gross findings were observed. Histologic evaluation identified a disseminated lymphoproliferative neoplasia in the lung, liver, kidney, spleen, heart, gastrointestinal tract, meninges, brain, skin, skeletal muscle, and bone marrow. Proliferative heterophilic pericarditis and myocarditis with intralesional bacteria was also identified. Neoplastic cells are immunoreactive for CD3 and negative for PAX5 and CD20. *Providencia spp*., *Citrobacter spp*., and *Klebsiella oxytoca* were cultured from the fresh pericardium.

DISCUSSION: Neoplasia, especially lymphoproliferative neoplasia, are likely underreported in Serpentes and infrequently subcategorized into T or B-cell origin. Bacterial pericarditis and myocarditis in this case are presumed secondary to immune suppression. To our knowledge, this is the first report of lymphoproliferative neoplasia in this species and of endocarditis associated with these bacteria species.

22: ESCHERICHIA COLI ASSOCIATED MALAKOPLAKIA IN THE KIDNEY AND URINARY BLADDER OF A 12-YEAR-OLD VIZSLA DOG

Raquel Rech, Paula Giaretta, Kathleen Aicher, Kellyn McNulty, Molly Haman, Haley Dodson
Texas A&M University, College Station, TX, USA

Malakoplakia is a poorly understood and uncommon granulomatous disease characteristically affecting the urinary bladder of veterinary species. The key histologic finding of diffuse sheets of foamy macrophages containing periodic acid-Schiff (PAS) inclusions is characteristic for the diagnosis of malakoplakia. Similar to granulomatous colitis of Boxer and French bulldogs, malakoplakia is believed to be associated with Escherichia coli infection, with a preference for females and younger animals. In people, malakoplakia is correlated with chronic inflammatory responses secondary to kidney transplants and infection with gram-negative bacilli such as E. coli. Here we present a case of a 12-year-old female spayed Vizsla dog with chronic weight loss, mixed bowel diarrhea, and recurrent urinary tract infections. Urine culture yielded Escherichia coli, and ultrasonic imaging of the kidneys and urinary bladder revealed bilateral chronic degenerative

nephropathy and an irregularly marginated bladder lumen with multifocal nodular proliferations. Postmortem examination revealed multifocal tan to yellow, irregular nodular regions within the renal medulla, and the urinary bladder was consistent with the previous imaging of numerous, tan to red, sessile nodules on the mucosa. Histology of both organs showed sheets of foamy Iba-1 positive macrophages with cytoplasmic granules and inclusions intensely PAS positive and occasional intracytoplasmic von Kossa and Prussian blue positive Michaelis-Gutmann inclusion bodies, consistent with malakoplakia. Fluorescence in-situ hybridization confirmed Escherichia coli within macrophages. To the best of our knowledge, this is the first case report of malakoplakia within the kidney of a dog.

23: MENINGOMYELOENCEPHALITIS BY LAGENIDIUM GIGANTEUM FORMA CANINUM IN A DOG

Matías Dorsch[1], Emma Carr[1], Colleen Embersics[1], Andrew Hanzlicek[2], Shannon Dehghanpir[1], Cesar Menk[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]MiraVista Diagnostics, Indianapolis, IN, USA

Background: *Lagenidium giganteum* is an oomycete that has been associated with cutaneous and subcutaneous lesions in dogs. Despite uncommon, those infections may be fatal.

Objective: To describe the gross, histologic, and molecular findings of a case of lagenidiosis in a dog.

Methods: Routine gross examination, histologic processing, PCR and sequencing were performed.

Results: A 4-year-old intact female Labrador Retriever was found dead after a clinical history of prostration, tetraparesis, dullness, opisthotonos, and anisocoria. The meninges on the ventral aspect of the pons, medulla oblongata, and cervical spinal cord extending to C3 were opaque and thickened up to 3mm, with coalescing areas of hemorrhage. On microscopic examination, the dura mater and leptomeninges surrounding the cervical spinal cord and brainstem were severely expanded by pyogranulomatous inflammation characterized by abundant macrophages and numerous multinucleated giant cells, degenerate and viable neutrophils, plasma cells and lymphocytes, all embedded in proliferating fibrous connective tissue. Within affected areas, there were abundant pale-staining, 8-12 µm wide, infrequently septate hyphae with thin, non-parallel walls, irregular branching, and clear bulbous swellings. The inflammation broadly extended into the white matter with multiple perivascular cuffings, myelin sheaths dilation, and neuronal necrosis. PCR and sequencing on a frozen section of the brainstem yielded a strong alignment with *Lagenidium giganteum* forma *caninum.*

Conclusions: Neurological disease caused by *L. giganteum* has been rarely reported in dogs. Lagenidiosis should be considered a differential diagnosis in canine patients with spontaneous, progressive neurological disorders.

24: KLEBSIELLA PNEUMONIAE INFECTION IN RACCOONS (PROCYON LOTOR): REPORT OF THREE CASES

Matías Dorsch[1], William Holl[1], Bianca de Cecco[1], Laura Peak[2], Pablo Fresia[3], Christine Walsh[2], Maria Mitchell[2], Marge Gill[1], Cesar Menk[1], Emi Sasaki[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]Louisiana Animal Disease Diagnostic Lab, Baton Rouge, LA, USA, [3]Unidad Mixta Pasteur + INIA, Institut Pasteur de Montevideo, Montevideo, Uruguay

Background: *Klebsiella pneumoniae* is a gram-negative bacterium associated with opportunistic respiratory, reproductive, and urinary infections, that may lead to fatal septicemia.

Objective: This study describes the gross, histologic, microbiological, and molecular features of three cases of klebsiellosis in raccoons (*Procyon lotor*).

Methods: Gross and histopathological examination, bacterial culture, and whole genome sequencing (WGS) were performed.

Results: Three 5-to-7-month-old raccoons died after exhibiting lethargy, anorexia, tachypnea, and diarrhea. Grossly, the peritoneal cavity contained up to 600 mL of opaque, white to yellow, viscous material (3/3). The omentum was diffusely dark red. Mesenteric lymph nodes were diffusely enlarged, and white viscous fluid oozed from the cut surface (2/3). Microscopically, the omentum and serosal surfaces of abdominal viscera were markedly infiltrated by neutrophils and foamy macrophages occasionally containing short, encapsulated, gram-negative rods, mixed with fibrin and necrotic debris. In the mesenteric lymph nodes, there was pyogranulomatous lymphadenitis with areas of necrosis, and numerous intracellular and extracellular gram-negative short rods. Other findings included ulcerative ileotyphlitis with intralesional bacteria (1/3), eosinophilic enterocolitis with intraluminal parasites (2/3), neutrophilic and histiocytic splenitis (2/3), and eosinophilic adrenalitis (1/3). Hypermucoviscous *Klebsiella pneumoniae* was isolated in pure culture from peritoneal swabs and lymph nodes (3/3). WGS from one isolate determined the strain type (ST60) and the presence of well-known virulence factors including capsule polysaccharide (*rmpA*), yersiniabactin (*ybt, irp1, irp2, fyuA*), and salmochelin (*iroB/C/D/N*).

Conclusions: Herein described is a fatal septicemic infection by *K. pneumoniae* in raccoons that, to our knowledge, has not been reported thus far.

25: PSEUDORABIES IN A HUNTING DOG

Matías Dorsch, Cesar Menk
Louisiana State University, Baton Rouge, LA, USA

Background: Domestic and wild swine are the natural hosts of Suid Herpesvirus-1 (SuHV-1), the causative agent of pseudorabies. Clinical disease in swine consists mainly of reproductive and respiratory signs, although wild hogs are mostly asymptomatic. Other species, including dogs, are accidental hosts that experience acute fulminant disease.

Objective: To describe the gross, histologic, and molecular features of a pseudorabies case in a dog.

Methods: Routine gross examination, histologic processing, and PCR for SuHV-1 were performed.

Results: A 3-year-old, female Plott hound canine died with a history of facial pruritus for a week following contact with a feral hog. Grossly, there were coalescing areas of cutaneous alopecia with subcutaneous hemorrhage and edema on the left frontal, temporal and maxillary regions. The tonsils were mildly enlarged. The lungs were congested with multifocal hemorrhages, and there was marked dirofilariasis in the heart. Microscopically, the trigeminal ganglion was infiltrated by lymphocytes and histiocytes. In the brainstem, glial nodules and lymphocytes were in the neuropil, and blood vessels were cuffed by lymphocytes and histiocytes. Multiple neurons in the trigeminal ganglion and brainstem were degenerated or necrotic, with rare satellitosis and neuronophagia. Affected neurons occasionally contained intranuclear glassy eosinophilic viral inclusions that peripheralized the chromatin. There were reactive lymphoid follicles with lymphocytolysis in the tonsils. SuHV-1 was detected by PCR in the brainstem.

Conclusions: Although pseudorabies has been eradicated in domestic pigs in the USA, the virus still circulates in wild boars across the country, thus posing significant threat to accidental hosts and commercial swine farms.

27: IMMUNOHISTOCHEMICAL AND PATHOLOGICAL INVESTIGATION OF AN UNDIFFERENTIATED OVARIAN CARCINOMA WITH SYSTEMIC METASTASES IN A PET CORN SNAKE (PANTHEROPHIS GUTTATUS)

Catherine Ennett[1], Mark Stidworthy[2], Carlo Bianco[1], Marta Gruarin[1], Sara Degl'Innocenti[1], Richard Lea[1], Sarah Cripps[1], Ana Ortiz[1]
[1]University of Nottingham School of Veterinary Medicine & Science, Sutton Bonington, United Kingdom, [2]International Zoo Veterinary Group, Keighley, United Kingdom

Background:

A 13-year-old female corn snake was presented for post-mortem examination after being found dead in its vivarium. An intracoelomic mass was identified at post-mortem examination in the region of the splenopancreas and right ovary, with pulmonary metastases. Cytology was performed. Histopathology revealed a neoplasm effacing the right ovary and histologically a presumptive diagnosis of undifferentiated carcinoma was made.

Objectives:

The aim was to characterize the histopathological and immunohistochemical features of an uncommon neoplasm in a corn snake, and to highlight the steps and challenges involved in the process of investigating tumor histogenesis in reptile species.

Methodology:

The following antibodies were tested on the neoplasm and on an ovary from a corn snake (control): Pancytokeratin and alfa SMA (polyclonal antibodies), and Cytokeratin, desmin, vimentin, p53, Melan A (monoclonal antibodies). To corroborate the interpretation of the immunohistochemistry, in silico cross reactivity was estimated when possible via protein-protein Basic Local Alignment Search Tool (BLAST) to assess the sequence similarity setting a cut off at 60%.

Conclusions:

The cross-reactive antibodies were: panCK, desmin (82.90% similarity) and alfa smooth muscle actin (99.73% similarity) and the neoplasm was positive for PanCK. In addition to describing an uncommon ovarian neoplasm in corn snake, this case also demonstrates the value of using in silico methods to aid in the interpretation of immunohistochemistry in species such as reptiles, in which immunohistochemistry is rarely used and where there is a paucity of available immunohistochemical markers.

28: BACTERIAL PNEUMONIA, PLEURITIS, AND MEDIASTINITIS WITH SPLENDORE-HOEPPLI PHENOMENON AND CONCOMITANT MEMBRANOUS GLOMERULOPATHY IN A 5-YEAR-OLD COMMON MARMOSET

Lindsey Ferguson, Alexis Roach, John Trupkiewicz
Johns Hopkins University, Baltimore, MD, USA

A 5-year-old female Common Marmoset (*Callithrix jacchus*) presented with four days of lethargy, hunched posture, squinting, and thin body condition, progressing to anorexia, hypothermia, and dehydration. Bloodwork revealed severe azotemia, elevated anion gap, moderate hypoglycemia, hyperkalemia, and anemia. Aggressive fluid therapy, heat support, and nutritional therapy were initiated, but the animal was found deceased the next day. At gross necropsy, the left lung was markedly expanded by innumerable ~1-3mm diameter, semi-firm, white nodules, with fibrinous pleural adhesions. The right lung was less severely affected. A mild reticulated pattern on the cortical surface

of both kidneys, vomitus around the mouth, and a missing upper left canine tooth were noted. Aerobic lung culture yielded few alpha-hemolytic *Streptococcus* spp. There was no growth on anaerobic culture. Histology of lung revealed severe necrotizing and pyogranulomatous bronchoalveolar pneumonia with intralesional filamentous organisms that were faintly gram-positive, acid fast negative, and argyrophilic, with Splendore-Hoeppli phenomenon. Additional histologic findings included lymphohistiocytic and suppurative mediastinitis, tracheobronchial lymph node hyperplasia and histiocytosis with scarring, and diffuse, bilateral, segmental membranous glomerulopathy. Based on the histologic findings and distinct morphotinctorial pattern, *Actinomyces* pneumonia is suspected (r/o *Nocardia* spp.), with secondary glomerulopathy due to immune complex deposition. *Actinomyces* spp. are normal flora in the oral cavity and GI tract, and opportunistic pathogens causing pyogranulomatous disease. *Actinomyces* and *Nocardia* can both induce Splendore-Hoeppli phenomenon and chronic progressive pulmonary infections. No other animals in the colony have exhibited similar signs. PCR was pursued for definitive diagnosis.

29: HYPERKERATOSIS AND NECROULCERATIVE DERMATITIS IN MULTIPLE GROWER PIGS FOLLOWING EXCESSIVE DIETARY MAGNESIUM INTAKE

Brett Fisher, Scott Fritz, Kristen Hill-Thimmesch
Kansas State University, Manhattan, KS, USA

Magnesium is an essential macromineral element with roles in a variety of homeostatic processes including oxidative phosphorylation, immunity, and nerve signal transduction. In growing pigs, supplemental magnesium may be provided in the diet as a method to reduce stress and improve meat character at the time of slaughter, or to ensure appropriate nutritional content. Acute toxicity from overt magnesium supplementation in pigs is rarely documented in veterinary literature but has been associated with dermal crusting and sloughing along the hind limbs and ventrum, putatively resulting from caustic irritation secondary to elevated urine or fecal pH following ingestion of excessive magnesium.

Approximately 40% of grower pigs on a mid-western farm acutely developed widespread dermal crusting and necrosis affecting the hind limbs, ventrum, and pinnae. Affected animals had been fed a ration containing markedly elevated magnesium levels based on feed analysis. Gross and histologic examination of multiple pigs revealed variably severe, multi-regional necroulcerative dermatitis and hyperkeratosis with evidence of gastrointestinal and urinary tract irritation. Alkaline urinary and fecal pH were documented, and differential diagnoses were investigated by a combination of feed analysis, biochemical evaluation, PCR assays, and liver trace mineral analysis. Ultimately, lesions were attributed predominantly to magnesium toxicosis.

This case illustrates a rare but potentially painful and costly disease arising from feed mis-formulation, which may resemble other dermal conditions of pigs. Magnesium toxicosis should be considered in cases of hind limb and ventrum hyperkeratosis and dermatitis in pigs and ruled out via analysis of feed and trace mineral analysis.

30: PERINEAL SQUAMOUS CELL CARCINOMA IN A SAANEN GOATS, IN CHILE

Carlos A. Flores Olivares[1,2], Carlos Ramírez[1], Daniel Cabre[3], Alejandra A. Covarrubias[1,2,4], Claudio Coddou[4]
[1]Facultad de Ciencias Agropecuarias, Universidad del Alba, La Serena, Chile, La Serena, Chile, [2]Laboratorio de Investigación en Ciencias Veterinarias (LiCiVet), Universidad del Alba, La Serena., La Serena, Chile, [3]PRODESAL, La Higuera, Chile, La Higuera, Chile, [4]Departamento de Cs. Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile

Background: SCC are malignant neoplastic processes originating from epidermal keratinocytes. This type of neoplasia has been reported in multiple species, such as felines, horses, cattle, and dogs, and

less frequently in pigs and goats. Our report presents a neoplastic episode in the perianal region in a group of adult Saanen goats in La Higuera, Chile. This geographical area is characterized by a warm climate with temperatures in summer reaching up to 38°C, high exposure to UV rays, and limited shade, where similar episodes are reported frequently.
Objectives: The study aimed to perform the surgical excision of the neoplastic lesion, make a histopathological diagnosis, and evaluate the characteristics of tumor behavior using different antibodies.

Methods: 70 goats, 7.14% (n=5) presented lesions in the perianal areas, characterized by multiple nodules, darkened, and irregular. Surgical excisional removal mass and samples were then fixed in 10% buffered formalin and stained with H&E, Masson's trichrome stain and analysis with antibodies and Immunofluorescent against Ki67, Pancytokeratin and Vimentin.

Results: Microscopic: Polygonal neoplastic cells and moderate pleomorphism which lose intercellular cohesion, emperipolesis, and form keratin pearls. From superficial to deep areas, polygonal to spindle-shaped cells proliferated, disrupting the basal membrane and infiltrating deep dermal and muscular tissue, generating an epithelial-mesenchymal transition. Mitotic count was up to 12 mitoses in 2.37 mm^2 with positivity for Ki67.

Conclusions: We demonstrated evidence of tumor proliferative activity based on Ki67 and modification in the cytoskeletal proteins of infiltrative neoplastic cells.

31: YEYUNAL GRANULOMA BY FILOBASIDIUM CHERNOVII ASSOCIATED WITH INTUSSUSCEPTION IN A 1.5-YEAR-OLD DOG

Carlos A. Flores Olivares[1,2], Julian Reyes[3], Pamela Thomson[4], Carlos González[5]
[1]Facultad de Ciencias Agropecuarias, Universidad del Alba, La Serena, Chile, [2]Laboratorio de Investigación en Ciencias Veterinarias (LiCiVet), La Serena, Chile, [3]Clínica Veterinaria Claudia Ojeda, La Serena, Chile, [4]Laboratorio de Microbiología Clínica y Microbioma, Escuela de Medicina Veterinaria, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile, [5]Laboratorio de Anatomía e Histopatología, Escuela de Medicina Veterinaria, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

Background: Granulomas associated with intussusception in dogs have been sporadically reported. In our case, we managed to identify the causal agent as *Filobasidium chernovii* previously classified as *Cryptococcus Chernovii.*

Objectives: To identify the causal agent of a granulomatous lesion in the jejunum associated with intussusception in a 1.5-year-old canine patient.

Methods: In a 1.5-year-old Belgian Shepherd dog with a history of obstruction and chronic diarrhea, underwent abdominal ultrasound, which revealed a jejunal mass. The affected segment was surgically removed, fixed, and subjected to histopathological analysis using H&E and PAS staining. DNA extraction from the sample was performed, followed by sequencing of the ITS1 and ITS2 regions

Results: A mass measuring 4.25 x 1.73 cm was identified, firm upon cutting, with an irregular surface, displaying heterochromatic areas of reddish, grayish, brownish, and whitish hues. Histopathological findings showed intestinal muscular tissue with multiple histio-neutrophilic inflammatory foci with multinucleated giant cells and numerous PAS-positive extracellular rounded yeast-like structures with thick capsules exhibiting budding figures, surrounded by bands of collagenous fibroconnective tissue. Sequencing results confirmed the presence of *Filobasidium chernovii.*

Conclusion The etiology of the granuloma associated with intussusception in this dog was confirmed to be caused by *Filobasidium chernovii*..

Similar cases in dogs have reported *Naganishia albida* (formerly Cryptococcus albidus) as the causal agent. Cryptococcosis is classified as a rare infection in dogs and is typically associated with *Cryptococcus gattii* and *Cryptococcus neoformans*, which can cause lesions in the central nervous system and fungemia.

32: ARTHRITIS AND INTERSTITIAL PNEUMONIA IN DAIRY GOATS IN CHILE

Carlos A. Flores Olivares, Carlos N. Pérez Cuello
Universidad del Alba, La Serena, Chile

Arthritis and interstitial pneumonia are significant health concerns in dairy goats in Chile. These conditions are often associated with various infectious agents, necessitating thorough diagnostic approaches. Serological tests for Mycoplasma spp, Caprine arthritis encephalitis (CAE), and Maedi Visna were conducted on an adult female Saanen goat presenting with asymmetric bilateral joint swelling and moderate to severe respiratory distress. Despite testing positive for CAE serologically, immunohistochemical analyses from tissues of the mammary gland, CNS, lungs, and joints were negative for CAE and Mycoplasma spp.

Necropsy findings revealed joint capsule thickening with discontinuity and abundant whitish to yellowish content. The lungs exhibited increased volume with symmetric bilateral costal indentation, a rubbery texture, and a heterochromatic pattern. The pericardial sac contained translucent yellow-amber fluid (30 ml), and the heart showed whitish areas predominantly in the left ventricle and papillary muscles, along with isolated myofibril degeneration and necrosis. Microscopic examination of joint tissues showed necrotic foci with basophilic granular accumulations surrounded by inflammatory cells, while the lungs displayed thickened septa with type II pneumocyte hyperplasia and interstitial edema.

PCR testing detected Mycoplasma spp in lung samples, suggesting its involvement. However, the negative results in key tissues suggest another potentially unidentified etiological agent. Therefore, further sequencing tests from obtained samples are recommended to elucidate the complete etiology of arthritis and interstitial pneumonia in dairy goats in Chile. Understanding these factors is crucial for effective disease management and prevention strategies in livestock.

33: MAST CELL TUMORS IN CANINES: 65 CASES

Carlos A. Flores Olivares[1], Constanza Rodríguez[2], Pablo Calderón[3], Milena Pérez[1], María Saldías[1]
[1]Universidad del Alba, La Serena, Chile, [2]Universidad Austral de Chile, Valdivia, Chile, [3]Universidad de Los Lagos, Osorno, Chile

This report details the classification of cutaneous mast cell tumors in dogs based on histological grade, location, age, breed, and gender. Out of 845 histopathological reports, 65 cases of mast cell tumors were identified, comprising 7.7% of the total. Among these cases, 43 (66.1%) were classified as low-grade mast cell tumors, 21 (32.3%) as high-grade, and 1 (1.5%) as subcutaneous mast cell tumor. Females accounted for 45 cases (69.2%), while males represented 20 cases (30.8%). The most common breeds diagnosed were mixed-breeds (36.9%), followed by Boxers (18.5%) and Poodles (6.2%). These tumors were typically found in dogs aged 3 to 15 years, with a peak occurrence between 6 and 8 years.

Mast cell tumors in dogs are classified using the Patnaik and Kiupel grading scales, where Kiupel distinguishes between low and high grades. However, research indicates that some low-grade tumors can behave aggressively. Therefore, it is recommended to supplement diagnosis with H&E staining

and additional techniques like c-KIT marker identification to better understand tumor behavior and guide treatment with tyrosine kinase receptor inhibitors. Recent studies also suggest assessing tumor location relative to growth pattern (cutaneous or subcutaneous).

Proposing a refined analysis based on c-KIT expression and specific diagnostic criteria could enhance treatment planning and improve outcomes for affected dogs. This approach aims to tailor therapies more effectively, potentially enhancing patient survival rates.

34: JUNCTIONAL EPIDERMOLYSIS BULLOSA IN A DOMESTIC SHORTHAIR CAT

Rebecca Fultz[1], Jorge Mendieta Calle[1], McKenna Snidow[2], Allison Watson[1]
[1]Department of Microbiology, Immunology, and Pathology, Colorado State University College of Veterinary Medicine, Fort Collins, CO, USA, [2]Department of Clinical Sciences, Colorado State University College of Veterinary Medicine, Fort Collins, CO, USA

A 10-year-old, male castrated, Domestic Shorthair cat presented to the dermatology department at Colorado State University for a chronic history of truncal and tail alopecia, pruritus, and onychodystrophy. Initially diagnosed with allergic dermatitis, superficial pyoderma and onychodystrophy, two months later the cat developed an oral mass. Periodontal treatment and biopsy of the mass was elected through CSU's dentistry department. Biopsy of the oral mass was collected, but remaining treatment was terminated due to severe sloughing of the oral mucosa. Biopsy revealed mild benign changes including epithelial hyperplasia and minimal inflammation. The cat acutely declined due to systemic hypertension and previously diagnosed respiratory disease with humane euthanasia and necropsy elected. Grossly, there were full thickness defects of the oral mucosa, distorted, discolored nail beds, and irregular, often absent nails on all four feet. Histologic evaluation of multiple sections of haired skin and mucosa demonstrated acanthosis and epithelial dissociation from the basement membrane with sub-basilar clefts that are either empty or filled with basophilic fluid and fibrin. Moderate lymphoplasmacytic and mastocytic inflammation dissected the subepithelium. PAS staining highlighted the basal epithelial dissociation. These findings are consistent with Junctional Epidermolysis Bullosa (EB), a rare vesiculobullous subepithelial autoimmune skin and mucosal disease. EB is characterized by defective adhesion of the epidermis to the dermis leading to fragility and blister formation. There are many subtypes reported in human literature, with differentiation requiring additional genetic analysis. EB has only been documented in a few cases in veterinary literature and represents a rare autoimmune dermatologic disease.

35: TUMOR-ASSOCIATED PULMONARY HYPERTENSION IN A WEST HIGHLAND WHITE TERRIER

Rachael Gruenwald[1], Carol Reinero[2], Aida Vientos-Plotts[2], Isabelle Masseau[3], Kurt Williams[1]
[1]Oregon State University, Corvallis, OR, USA, [2]University of Missouri, Columbia, MO, USA, [3]University of Montréal, Saint-Hyacinthe, QC, Canada

Tumor-associated pulmonary hypertension (TAPH) is a rare form of pulmonary hypertension in humans associated with malignancy in the lungs. Vascular histologic features associated with TAPH are: 1) pulmonary tumor microembolism (neoplastic cells in alveolar capillaries, arterioles and venules) 2) pulmonary tumor thrombotic microangiopathy (pulmonary arterial and venous fibrointimal proliferation associated with neoplastic cells) and 3) pulmonary tumor macroembolism (large pulmonary arteries plugged with neoplastic cell aggregates). In the current case, an 11-year-old West Highland White Terrier was referred to the University of Missouri Veterinary Health Center for syncope. Thoracic computed tomography and airway cytology revealed metastatic pulmonary carcinoma and echocardiography supported severe pulmonary hypertension. After progression of clinical signs, the dog was euthanized. The right cranial, middle and caudal lung lobes were collected, cannulated via the lobar bronchus and inflated with 10% neutral buffered formalin and submitted to the Oregon Veterinary Diagnostic Laboratory for evaluation. Multiple sections of each lung lobe were

routinely processed and stained with hematoxylin-eosin. Verhoff-Van Gieson histochemistry was used to distinguish arteries and veins. Immunohistochemistry for cytokeratin was used to co-localize neoplastic epithelial cells with vascular remodeling. Variably sized masses comprised of neoplastic epithelial cells were present in all lung lobes. Pulmonary tumor microembolism and arterial and venous remodeling consistent with pulmonary tumor thrombotic microangiopathy were widespread in all lobes. Pulmonary tumor macroembolism was not noted. Herein we present the first evidence for tumoral pulmonary hypertension in a dog. These findings suggest the need for further characterization of this syndrome in veterinary medicine.

36: MELANOCYTE AND MAST CELL-RICH THYMOMA WITH SUSPECTED PRECURSOR-TARGETED IMMUNE-MEDIATED ANEMIA (PIMA) IN A DOG

Taylor A. Hanson, Miranda D. Vieson, Marissa Goodwin, Timothy M. Fan, Michael F. Rosser
University of Illinois, Urbana, IL, USA

Background:

A 12-year-old, male neutered Labrador Retriever was referred to the University of Illinois for a cranial thoracic mass (measuring 10.0cm x 6.4cm) and a moderate, non-regenerative anemia (hematocrit 16.2%).

Results:

On cytologic evaluation of the mass, mast cells were the predominant cell type. A small population of mixed lymphocytes was also present. These findings raised initial suspicion for a metastatic mast cell tumor within a mediastinal lymph node, although an unusual mast cell-rich thymoma was not excluded. Moreover, no primary lesions could be identified to support a clinical diagnosis of metastatic mast cell disease. Cytologic evaluation of bone marrow revealed ineffective erythroid hyperplasia with maturation arrest (suspicious for PIMA), and lymphocytosis. Excisional biopsy of the thoracic mass confirmed a diagnosis of thymoma, though the lesion was heavily infiltrated with both well-differentiated mast cells and melanocytes (suspected to be non-neoplastic).

Conclusion:

This was an interesting presentation of thymoma in several regards. Although thymomas typically contain variable numbers of mast cells, the predominance of these cells on cytology was unusual. The melanocytic infiltration identified histologically was also atypical; thymomas with a melanocytic component have been rarely reported in dogs and humans, though prognostic implications remain unclear. Thymomas are commonly associated with a variety of autoimmune complications, but PIMA has only rarely been described. Still, thymoma was considered the most likely inciting cause of PIMA in this case. Resolution of anemia after resection of the mass could unfortunately not be confirmed as the patient was euthanized shortly after surgery.

37: MAMMARY GLAND ADENOCARCINOMA IN TWO GOATS

Luan Henker, Laura Lee, Katharine Horzmann
Auburn University, Auburn, AL, USA

Mammary gland neoplasms are uncommon in ruminants. We report two cases of mammary gland adenocarcinoma in goats. Goat 1 was a 10-year-old mixed-breed goat, and Goat 2 was a 13-year-old Nubian goat. Both goats had mammary gland enlargement and were euthanized due to poor prognosis. Grossly, Goat 1 had a 13-cm x 8-cm x 7-cm, white to tan, predominantly solid, firm, and encapsulated mass in the left mammary gland. A similar 15-cm x 8-cm x 7-cm, multilobulated, encapsulated mass was in the pelvic cavity extending to the abdominal cavity, firmly attached to the

lumbar vertebral column (metastasis). The vertebral body of L6 had evidence of bone lysis. Grossly, the right mammary gland of Goat 2 was markedly enlarged at 8-cm x 7.5-cm x 30-cm. On the cut surface, this mammary gland was white to tan, firm, and contained numerous cystic cavitations filled with light green fluid. Histologically, both cases were diagnosed as mammary gland adenocarcinoma. Intra-abdominal and vertebral metastasis were confirmed in Goat 1. Goat 2 had no evidence of metastasis. In both cases, neoplastic epithelial cells were arranged in pluristratified irregular tubules and nests; neoplastic cells had moderate to marked anisocytosis and anisokaryosis, and the mitotic count was twenty-four (Goat 1) and twenty-two (Goat 2) in 2.37mm^2. In Goat 2, multifocal lobules were centered on areas of necrosis, and both cases had extensive desmoplasia. Mammary gland neoplasia should be a differential for mammary gland enlargements in older goats.

38: BICAVITARY MESOTHELIOMA IN A DONKEY (EQUUS ASINUS)

Shauna Hilton[1], Gail Leeming[2], Catherine Ennett[1], Jonathon Ruiz[3], Ana Ortiz[1,4]
[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonnington, United Kingdom, [2]School of Veterinary Science, University of Liverpool, Neston, United Kingdom, [3]Oakham Veterinary Hospital, Oakham, United Kingdom, [4]VPG Histology, Bristol, United Kingdom

Background

Mesotheliomas are considered rare and affect many animal species but have not been reported in donkeys (*Equus asinus*).

Objectives

Here we report a case of peritoneal and pleural epithelioid mesothelioma in a donkey.

Methods

A 33-year-old Jenny presented with weight loss, depression, anorexia. Physical exam revealed tachycardia, ileus, and delayed capillary refill time. The animal was euthanized and submitted for necropsy. Macroscopic examination revealed cachexia, 24.5L serosanguinous peritoneal effusion, 500ml serosanguinous pleural effusion, and multifocal to coalescing exophytic, papillary, white, occasionally hemorrhagic, pinpoint to 5cm x 5cm x 2cm nodules multifocally on pleural and peritoneal surfaces and occasionally infiltrating into viscera.

Results

Histopathology of the omentum revealed an infiltrative, expansile, unencapsulated, poorly demarcated, moderately cellular neoplasm composed of cuboidal to polygonal cells arranged in acini, tubules and nests, and rarely micropapillary projections, on a moderate fibrovascular stroma. Neoplastic cells had variably distinct cell borders; abundant eosinophilic granular to indistinctly vacuolated cytoplasm; a pleomorphic, central nucleus with finely stippled to marginated chromatin; and up to 3, occasionally very large distinct magenta nucleoli. There was marked anisocytosis and anisokaryosis and frequent mitoses.

Neoplastic cells exhibited diffuse strong vimentin immunolabelling, and most neoplastic cells have moderate immunolabelling for cytokeratin.

Conclusions

Macroscopic, histopathologic and immunohistochemical examinations were consistent with a bicavitary epitheloid mesothelioma, a rare neoplasm in domestic animals which has not been

reported in the donkey. Dual vimentin and cytokeratin immunopositivity can also be found in ovarian or renal carcinomas but no evidence of a primary carcinoma was identified.

39: SYSTEMIC TOXOPLASMOSIS IN SPEKE'S GAZELLES (GAZELLA SPEKEI)

William Holl[1], Robert MacLean[2], Luis Tondo[1], Jeongha Lee[1], Fabio Del Piero[1]
[1]Louisiana State University, Baton Rouge, LA, USA, [2]Audubon Zoo, New Orleans, LA, USA

Background: Toxoplasma gondii is an obligate, intracellular, apicomplexan protozoan which can cause systemic disease in many animal species. Felids are the definitive hosts with the organisms having a typical enteroepithelial life cycle resulting in the oocyst release into the environment. Once these oocysts sporulate and are consumed by an intermediate host, the sporozoites excyst in the intestines and transform into tachyzoites causing protozoemia and then disseminate to multiple organs forming cysts containing bradyzoites. Disease in intermediate hosts is often associated with immunocompromised or immunosuppressed animals as cell-mediated immunity is an important factor in keeping the infection under control, although, several species have an increased susceptibility with higher mortality rates.

Case Description: Over a month, two out of four Speke's gazelles in the zoo died. The initial animal to succumb had a two-week history of intermittent diarrhea prior to becoming obtunded. Thoracic radiographs revealed a suspected pneumonia; however, the animal died during recovery from anesthesia. On postmortem microscopic examination, there was necrotizing and lymphohistiocytic, interstitial pneumonia with intralesional tachyzoites expressing T. gondii microneme protein MIC3 on immunohistochemistry. The remaining animals were serum negative for T. gondii; however, an additional Speke's gazelle was found dead on exhibit without premonitory signs. This animal had similar histologic pulmonary lesions with many zoites in multiple organs such as the liver, spleen, and lymph nodes. No bacteria were observed or grown on aerobic culture of either lung specimen.

Summary: These cases represent T. gondii causing systemic infection and death in Speke's gazelles.

40: A CANINE RIGHT ATRIAL MESENCHYMAL NEOPLASM: IT'S NOT WHAT YOU THINK

William Holl, Syon Link, Henry Green, Emi Sasaki
Louisiana State University, Baton Rouge, LA, USA

History: A 6-year-old, castrated Pitbull presented for recurrent ascites and suspected right-sided congestive heart failure. On examination, heart sounds were muffled and point of care ultrasound and radiographs revealed pleural and peritoneal effusions. The dog was transferred to the cardiology service the following day where an echocardiogram revealed a large, mixed echogenic mass occupying the right atrium which restricted tricuspid valve motion and caused a relative stenosis. Given the poor prognosis, the owners elected for humane euthanasia and necropsy.

Description: Most of the right atrial lumen was occupied by a firm, mottled red and tan, papilliferous mass with many pale tan striations on the cut surfaces measuring 5.5 x 5.5 x 3.5 cm. The mass extended from the wall, partially obstructed the caval entrance, and pressed against the tricuspid septal leaflet. On microscopic examination, neoplastic cells were arranged in streams and bundles on a myxomatous to collagenous stroma that variably stained positive for Alcian blue (pH 2.5). Neoplastic cells were spindle to stellate with indistinct cell borders and contain minimal eosinophilic, fibrillar cytoplasm and round to elongate, centrally placed nuclei with finely stippled chromatin and 1-2 nucleoli. Anisocytosis and anisokaryosis were moderate, and there were 8 mitoses in 2.37 mm2.

Summary: Based on the characteristics of this neoplasm, an intracardiac right atrial myxoid fibrosarcoma was diagnosed which resulted in right-sided congestive heart failure secondary to obstructing closure of the tricuspid valve. Primary cardiac fibrosarcomas represent malignant transformation of spindle cells and are considered rare in dogs with scattered reported cases.

41: MALAKOPLAKIA IN A YOUNG CAT

Sophie Hopkins, Ryan Jennings
Ohio State University, Columbus, OH, USA

Malakoplakia is an uncommon inflammatory condition that most often affects the urogenital tract of immunocompromised individuals. A stray young adult female domestic shorthair cat presented with dehydration, a body condition of 1/9, and firm, small, irregular kidneys that were painful on palpation. The cat was anemic with a packed cell volume of 19% and a total protein of 6.6 g/dL. Chronic kidney disease was suspected as the primary differential. Due to the poor prognosis, the patient was euthanized and submitted for a postmortem examination.

Gross evaluation confirmed small, firm kidneys with multifocal flat depressions in the capsular surface. Focally within the apex of the urinary bladder was a tan, well demarcated, raised, irregular plaque measuring 1.0cm x 1.5cm. Histopathology of the urinary bladder identified marked expansion of the lamina propria by diffuse infiltrates of histocytes with abundant granular eosinophilic cytoplasm consistent with von Hansemann-type macrophages which contained numerous intracytoplasmic basophilic inclusions of various sizes (Michaelis-Gutman bodies). The Michaelis-Gutmann bodies were positive for periodic acid-Schiff. This finding is consistent with the diagnosis of malakoplakia.

Malakoplakia is a rare disease in veterinary species, first identified in pigs. Since 2008, there have been three documented reports in the urinary bladders of cats and 8 reported cases in dogs. The histologic appearance is similar to canine granulomatous colitis seen in Boxers and French Bulldogs suggesting a possible deficient immune response to bacterial infection. Malakoplakia should be considered as a differential in young animals that present for dysuria that do not respond to antibiotic treatment.

42: TRANSEPIDERMAL CYTOTOXIC DERMATITIS WITH CONCURRENT INFILTRATIVE HISTIOCYTIC SARCOMA IN AN AGED C57BL/6 MOUSE

Bethany N. Howard, Katherine N. Gibson-Corley, Tzushan Sharon Yang
Vanderbilt University Medical Center, Nashville, TN, USA

An approximately 2-year-old male, experimentally naïve, wild-type C57BL/6 mouse presented with scaling dermatitis of the feet, tail, and face, hunched posture, and lethargy. Necropsy revealed moderate to marked multifocal lymphadenopathy and splenomegaly along with crusting, ulceration with subcutaneous edema of the entire head, face, neck, and all extremities. Histologically, liver showed diffuse infiltration by pleomorphic neoplastic histiocytes (immunolabeled with Iba-1) disseminated throughout the sinusoids, surrounding central and portal veins, and partially occupying multiple vascular lumens. The splenic parenchyma, multiple internal lymph nodes, and bone marrow were also diffusely expanded and effaced by the neoplastic histiocytes. These findings are suggestive of infiltrative form hepatic histiocytic sarcoma (HS) with multiorgan metastases. Regionally extensive in the skin, there was transdermal keratinocyte apoptosis with extensive epidermal detachment and necrosis, accompanied by neutrophilic and lymphoplasmacytic interface dermatitis, consistent with Stevens-Johnson Syndrome (SJS)/erythema multiforme (EM)/toxic epidermal necrolysis (TEN). While hepatic HS is a common finding in aged C57BL/6 mice, the connection between HS and skin lesions in this case remains unclear, as spontaneous SJS/EM/TEN lesions in rodents are not well-described. Considering the animal’s experimental status, it significantly reduces the probability of a drug-induced hypersensitivity. The skin lesion observed may potentially be a paraneoplastic manifestation of the

HS, although the possibility of other etiologies, such as bacterial or viral infections should also be considered.

44: DEVELOPMENT OF SPECIES IDENTIFICATION KEYS OF HAIR MORPHOLOGY OF MAMMALS OF TAIWAN USING THE LIGHT MICROSCOPE AND SCANNING ELECTRON MICROSCOPE

Ching-Yun Hsu, Wei -Hsiang Huang, Yi-Fan Jiang
Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background

Hair is a characteristic feature of mammals. Its polymorphism, resistance to adverse conditions, and ease of collection make it ideal for species identification. The study of hair morphology is widely applied in forensic science, ecology, and archaeology. Beyond the use of light microscopes, the scanning electron microscope (SEM) featuring high resolution and depth of field has also been employed in trichomorphology. Despite the existence of many hair atlases and identification keys targeting mammals from different regions worldwide, a systematic analysis of mammalian hair morphology in Taiwan is lacking.

Objective

The study aims to establish a hair database for terrestrial mammals of Taiwan and to construct an identification key by analyzing the characteristics of hair with optical microscopy and SEM.

Methods

Quantitative and qualitative analyses of cuticle, medulla and other features of hair using the light microscope were conducted on dorsalateral scapular guard hairs collected from 30 mammal species of Taiwan. SEM examination was also conducted in select species.

Results

Quantitative features, including hair length, maximum width, and medullary index, as well as qualitative features like cuticular patterns and medullary patterns, exhibited varying degrees of phylogenetic relationships among species. Distinct morphology of the root, the base and the tip of hair were observed among species. Variations of the interscale distance along the hair also differed and could aid in identification.

Conclusions

By integrating these hair characteristics, we have successfully established a hair morphology identification key for 30 terrestrial mammals of Taiwan, demonstrating a simple and cost-efficient method of species identification.

45: PATHOLOGY AND LOCALIZATION OF AVIAN REOVIRUS IN POULTRY WITH VIRAL TENOSYNOVITIS

Cheng-Shun Hsueh, Pablo Piñeyro, Olufemi Fasina, Mohamed Medhat El-Gazzar, Yuko Sato
Iowa State University, Ames, IA, USA

The re-emergence of Avian reovirus (ARV) is associated with various conditions, including arthritis/tenosynovitis, hepatitis, myocarditis, encephalitis, and enteritis. Despite extensive research, comprehensive histopathologic and viral antigen distribution of ARV-associated tenosynovitis are scarce. This study elucidates the pathologic features of ARV tenosynovitis, coupled with in situ

hybridization (RNAscope) to localize ARV M1 transcripts in lesions. Inclusion criteria for this study were legs from two chicken flocks (5-10 weeks old; 37 legs) and two turkey flocks (17-30 weeks old; 12 legs) submitted to ISU-VDL for necropsy with a clinical history of lameness, positive ARV qRT-PCR results (ranging from 21.7-26.5), and histologic lesions consistent with ARV. Gross findings included serous-to-viscous fluid within swollen joints in all flocks (4/4, 100%); affecting 18/37 (48.6%) chicken legs and 6/12 (50%) turkey legs. Hemorrhagic deep digital flexor and gastrocnemius tendons were seen in both chicken flocks (9/37; 24.3% of legs). Histologically, tenosynovitis (4/4, 100%) was characterized by predominant infiltrates of lymphocytes, plasma cells and macrophages in the synovial intima and subintimal fibrous tissues, and frequent perivascular lymphocytic nodules (3/4, 75%). The subintima stroma was usually expanded by fibrous tissue with neovascularization (3/4, 75%). The synovial membrane was thickened and papillated by hyperplastic synoviocytes (4/4, 100%). RNAScope revealed viral transcripts in the synovial subintimal fibroblasts of affected synovium (4/4, 100%). Other infectious causes were ruled out by histopathology, bacterial culture, and Mycoplasma PCR. This study provides the first report highlighting the detection of ARV transcripts within subintimal fibroblasts in lesions, offering insights into ARV diagnostics and pathogenesis.

46: SUBCUTANEOUS AMELANOTIC MELANOMA WITH METASIS TO SPLEEN AND LIVER IN A CANINE

Jennifer Hu, Jung Lee, Mary White, Kara Thomas, Joni Johnson
Midwestern University, Glendale, AZ, USA

An approximately three-year-old female Pit bull mix, recently adopted, presented for a pre-operative examination prior to ovariohysterectomy. At that time, the owner reported unexplained weight loss over four months. Preoperative examination revealed a large intra-abdominal mass suspected to be associated with the spleen or liver and a subcutaneous perivulvar mass. Cytology identified a neoplastic population with microvesiculated cytoplasm in both the perivulvar mass and rare to occasionally in the intraabdominal mass. Patient was sent to abdominal exploration under general anesthesia with ovariohysterectomy and an enlarged, cavitated spleen, a small mass noted on liver, and perivulvar subcutaneous mass were sent for histopathology. Histopathology revealed polygonal to pleomorphic neoplastic cells with occasional clear, distinct, intracytoplasmic vacuoles positive for vimentin on splenic, hepatic, and subcutaneous masses. Oil Red O on the splenic mass was positive for intracytoplasmic vacuoles within neoplastic cells. While SOX-10 of neoplastic cells was negative, melanoma was suspected since neoplastic cells were positive for Melan-A and an immunohistochemical cocktail for antibodies against Melan-A, PNL2, TRP-1, and TRP-2. Electron microscopy performed on the splenic mass revealed melanosomes and premelanosomes. These findings collectively confirmed a diagnosis of melanoma involving the spleen, perivulvar subcutis, and liver. This case represents a unique and diagnostically challenging case of amelanotic melanoma. The primary site is perivulvar subcutis with metastasis to the spleen and abdominal viscera, an extremely rare occurrence.

47: TUMOROUS LIPID PNEUMONIA IN A 10-MONTH-OLD DOMESTIC CAT

Mari Inohana[1], Carol Reinero[2], Aida Vientos-Plotts[2], Jason Eberhardt[2], Isabelle Masseau[3], Kurt Williams[1]
[1]Oregon State University, Corvallis, OR, USA, [2]University of Missouri, Columbia, MO, USA, [3]University of Montreal, Saint-Hyacinthe, QC, Canada

Endogenous lipid pneumonia (EnLP) can develop from obstructive airway disease preventing clearance of surfactant lipids. Small incidental foci of EnLP are common in adult cats, mutelids, and laboratory rodents. In the current case, a 10-month-old American domestic shorthair cat presented to the University of Missouri Veterinary Health Center following a 3-month period of cough and labored respiration. No mineral oil or other lipids had been administered. Thoracic imaging identified

pulmonary nodules and a patchy alveolar-interstitial pattern. Pyogranulomatous inflammation was noted on BAL cytology. Lack of response to empiric therapy with disease progression led to euthanasia. A necropsy was performed. Numerous large, raised, well-demarcated pale yellow-gold masses, up to 2.5cm, were present in all lobes. Sections of lung were fixed in 10% neutral buffered formalin and submitted to the Oregon Veterinary Diagnostic Laboratory for evaluation. Fixed lung was routinely processed and stained with hematoxylin-eosin and oil red-O. Histologically, all masses were comprised of numerous macrophages and neutrophils filling alveolar spaces. The cytoplasm of many macrophages contained fine vacuoles identified as lipid with oil red-O histochemistry. The interstitium between alveoli was expanded by similar cells and numerous spindle cells. Bronchioles were difficult to identify in the masses. Immunohistochemistry for smooth muscle actin was used to identify respiratory bronchioles. Respiratory bronchiolar smooth muscle was identified in normal lung and at the periphery of masses, but rarely within the masses. The young age of the cat and paucity of small airways associated with the masses suggests abnormal airway development leading to early-onset severe EnLP.

48: SPONTANEOUS VENTRUM DERMATITIS IN MICE WITH FVB/N BACKGROUND

Alexis Johnson[1], Michael McKinney[2], Jessica Wong[3], Katherine Gibson-Corley[2], Tzushan S. Yang[2]
[1]University of Tennessee, Knoxville, TN, USA, [2]Vanderbilt University, Nashville, TN, USA, [3]The Jackson Laboratory, Sacramento, CA, USA

Over the course of one year, six mice of FVB/N strain and transgenic mice on a FVB/N background developed a moderate to severe dermatitis with alopecia and disseminated ulcerations. The lesions were primarily confined to the ventrum and limbs with occasional extension up the flank. Histologically, lesions were characterized by chronic-active, perivascular to interstitial lymphoplasmacytic and neutrophilic dermatitis with acanthosis, mucinosis, edema, and furunculosis. Areas of ulcerations were frequently capped with a thick serocellular crust and colonies of mixed bacteria. In some cases, rare apoptotic keratinocytes were noted adjacent to regions of dermal necrosis and deep ulcerations. Affected mice included four females and two males housed across three different facilities. All mice were unrelated, experimentally naïve, and were adult breeders except two for which the breeding history was unknown. Given that cage mates of affected individuals were free of lesions, and no excluded pathogens (including ectromelia virus, fur mites, and *Corynebacterium bovis*) were detected within the institution, an infectious cause is considered less likely. Other strains of mice housed in the same rooms were also unaffected. The cause of these lesions remains unclear, but the characteristic lesion distribution and shared genetic background across affected animals is suggestive of a spontaneous dermatitis related to the FVB/N background strain. The potential that the dermatitis is due to an unidentified allergen or spontaneous genetic mutations is considered and requires further investigation.

49: A CASE OF THYMIC CARCINOMA WITH MULTI-CYSTIC STRUCTURES IN A NEW ZEALAND WHITE RABBIT (ORYCTOLAGUS CUNICULUS)

Jui-Ling Ku[1], Jo-Wen Chen[2], Wei-Hsiang Huang[1]
[1]National Taiwan University, Taipei, Taiwan, [2]Jong-Shing Animal Hospital, Kaohsiung, Taiwan

An 8-9-year-old male castrated rabbit presented with 2-3 months of dyspnea. A CT scan identified an anterior mediastinal mass adhering to the pericardium. Thoracotomy and partial pericardium mass excision were performed. Prior to surgery, 22 ml of pink pleural effusion was withdrawn, and post-surgery, 28 ml of dark red turbid liquid was extracted from the mediastinal mass. The specimen was a large cystic structure with heterogeneously beige and white walls, containing dark red fragile contents. Histological examination with hematoxylin and eosin staining and immunohistochemistry for cytokeratin (CK), CD3, and PAX5 was performed on formalin-fixed-paraffin-embedded tissue sections. Microscopically, the mass was a multicystic neoplasm with cyst walls lined by neoplastic squamous epithelial cells, exhibiting distinct cell borders and occasional keratin tonofilaments. Other

notable features included marked anisocytosis and anisokaryosis, multinucleated neoplastic cells, neoplastic giant cells, karyomegaly, and bizarre nuclei. The mitotic count was 7 per 10 HPFs (2.37 mm^2). Tumor emboli, capsular invasions with desmoplasia, and inflammation with cholesterol clefts were observed. Intraluminal dark red material, mainly lymphocytes (CD3 positive) and scattered epithelial cells (CK positive), suggesting the origin of thymic tissue. Based on these findings, the diagnosis was thymic carcinoma, further classified as the squamous cell carcinoma (SCC) subtype. Due to persistent pleural effusion, the patient was euthanized. This case represents an extremely rare instance of cystic thymic carcinoma or thymic carcinoma with concurrent cysts in a rabbit.

50: DISSEMINATED HISTIOCYTIC SARCOMA IN A CRAB-EATING MONGOOSE (HERPESTES URVA)

Jui-Ling Ku[1], Chiu-Hung Cheng[2], Pao Jung Wang[2], Yen-Hsueh Lai[2], Chian-Ren Jeng[1], Yen-Chen Chang[1]
[1]National Taiwan University, Taipei, Taiwan, [2]Taipei Zoo, Taipei, Taiwan

A 12 year-old female crab-eating mongoose, who had chronic kidney disease and squamous cell carcinoma located at the forehead, tumbled and was unable to walk with elevated BUN and creatinine prior to the death. There were several bulging masses at the right neck and the bilateral renal cortex appeared hyperechoic under ultrasound examination. At necropsy, four subcutaneous masses in different sizes were noted in the submandibular region, ranging from 1.9 x 0.7 x 0.3 to 3.5 x 3 x 1.5 cm. Multiple white nodules in varying sizes were scattered in multiple viscera. Many pinpoint white lesions were found in the cortex of bilateral kidneys. Microscopically, the round neoplastic cells with moderate to severe degrees of anisocytosis and anisokaryosis are arranged in sheets and observed in the submandibular lymph nodes, heart, urinary bladder, adrenal gland, adipose tissue adjacent to the ovary, and the anterior pituitary gland. Marked interstitial nephritis with fibrosis is noted multifocally in bilateral kidneys. The immunohistochemistry result demonstrated that neoplastic cells showed membranous and cytoplasmic immunoreactivity to ionized calcium-binding adapter molecule 1 (IBA1), while negative for multiple myeloma oncogene 1 (MUM-1) and pancytokeratin, suggestive of the histiocytic origin. The diagnosis of disseminated histiocytic sarcoma (HS) was given based on the immunohistochemistry result and multiple internal organ involvements without cutaneous lesion. The present case is first reported disseminated HS in crab-eating mongoose. The submandibular lymph node, the largest mass, was considered as the primary site due to the size, and the metastatic sites were uncommonly seen.

51: SUPRASELLAR GERM CELL TUMOR IN A YOUNG PITBULL DOG

Shanny H. Kuo, Miranda D. Vieson
University of Illinois Urbana-Champaign, Urbana, IL, USA

Background: Germ cell tumors usually form in the gonads but can also appear in the midline of brain as primary extragonadal tumors. In dogs, these tumors are rare and typically occur in young adults, mainly in the suprasellar region. Here we describe the morphologic and immunohistochemical characteristics of a differentiated suprasellar germ cell tumor in a young male PitBull dog.

Case presentation: A 3-year-old castrated male Pitbull was brought to the University of Illinois Neurology Service due to progressive behavior changes. Magnetic resonance imaging identified a large, round, delineated mass at the level of the thalamus. At necropsy, a 2.5 x 3.5 x 2.0 cm, soft, variegated brown and beige mass was found in the hypothalamic area dorsal to the sella turcica. Histopathology reveals a non-encapsulated, ill delineated, and infiltrative neoplasm affecting the thalamus, hypothalamus, and pituitary gland. It comprises proliferating round cells arranged in solid sheets and nests supported by a fine fibrovascular stroma (germinomatous appearance). Multifocally the neoplasm shows teratomatous differentiation into hepatoid cells (hepatoid-like pattern), secretory

glandular (acinar pattern), and squamous elements with rare evidence of cilia. Mitotic index is high in the round germinomatous cells. Immunohistochemistry revealed strong cytokeratin reactivity in tubulo-acinar structures, with germinomatous cells being immunonegative.

Conclusion: Differential diagnoses for sellar region brain tumors in dogs include pituitary tumors, craniopharyngioma, and suprasellar germ cell tumors. In this case, the midline location, immunohistochemistry, and histomorphology with mixed germinomatous and teratomatous differentiation supported a diagnosis of a suprasellar germ cell tumor.

52: NECROHEMORRHAGIC PNEUMONIA FROM EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI IN FOUR DOGS

Emily Swan[1], Elise LaDouceur[1], Sarah Sulkosky[1], Erin Hennessey[2], Richard Brooksby[2], Michael Lagutchik[2], Bruce Williams[1]
[1]Joint Pathology Center, Silver Spring, MD, USA, [2]Department of Defense Military Working Dog Veterinary Service, Joint Base San Antonio, TX, USA

Background: Extra-intestinal pathogenic *E. coli* (ExPEC) is a commensal organism that possesses virulence factors that may cause extra-intestinal disease. Disease from ExPEC is a concern in group-housed animals, such as laboratory animals. Although ExPEC is capable of causing rapid death, it is unclear why disease is sporadic with some animals in close proximity of infected animals remaining asymptomatic or even uninfected.

Clinical histories: In 3 years, four dogs housed individually in kennels in Texas had rapidly progressive respiratory distress. They were febrile (103.3-105.5^0 F) with increased to muffled bronchovesicular sounds. Thoracic radiographs revealed alveolar to interstitial pulmonary patterns (n=4), pneumothorax (n=2), and pleural effusion (n=2). Labwork revealed neutropenia (n=4) and prolonged clotting times (n=2). Three dogs were euthanized within 1 day, and one had cardiac arrest during a thoracotomy 3 days after onset of clinical signs. At necropsy, the thorax contained 200-300 mL of hemorrhagic fluid (n=2) and lung lobes were purple and firm (n=4). Hemorrhage was noted in the endocardium (n=2), meninges (n=1), and gastric lumen (n=1).

Results: Bacterial lung cultures (n=4) yielded *E. coli.* Histologically (n=4), pulmonary alveoli and small bronchioles were filled with hemorrhage, fibrin, edema, neutrophils, necrotic debris, and 1-2 µm, gram negative bacilli, consistent with *E. coli.* Cytotoxic necrotizing factor-1 was detected with polymerase chain reaction, consistent with ExPEC.

Conclusions: Pneumonia caused by ExPEC is sporadic, but is often associated with exposure to infected autologous, allogeneic, or xenogeneic (including human) feces. The pathogenesis is not fully understood and disease is often correlated to recent transport.

53: SERIAL THANATOTRANSCRIPTOMIC ANALYSIS OF CASPASE-3 IN MURINE CARDIAC MUSCLE TISSUE USING IN SITU HYBRIDIZATION (ISH) AND ITS CORRELATION TO NEXT-GENERATION SEQUENCING (NGS): A NOVEL APPROACH FOR ESTIMATING POSTMORTEM INTERVAL (PMI)

Pin-Ya Lai, Wei-Hsiang Huang, Yi-Hsiang Huang
Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Background: The estimation of the postmortem interval (PMI) poses a significant challenge in forensic medicine. Various approaches for determining PMI through postmortem changes have been proposed but often suffer from inaccuracies. In recent years, research has shifted towards molecular biology, particularly the thanatotranscriptome, which examines postmortem mRNA transcripts to

establish more precise PMI estimation methods.
Objectives: This study aims to observe *Caspase-3* expression using in situ hybridization (ISH) and its correlation to next-generation sequencing (NGS) in murine cardiac muscle.
Methods: We evaluated the thanatotranscriptomic expression of *Caspase-3* in the cardiac muscle tissue of sixty mice stored separately in 25℃ still-air chambers at 0, 2, 4, 8, 12, 18, 24, 30, 36, and 48 hours after death. The transcriptomes at 10 time points were examined using NGS, and *Caspase-3* was chosen as a pilot gene to study the morphological mRNA expression.
Results: Our results indicated that mRNA quality remained stable up to 24 hours postmortem in cardiac muscle tissue, allowing for continued NGS analysis. ISH results showed *Caspase-3* signals mainly in the cytoplasm, with frequent positive signals within the nuclei. Both ISH and NGS results showed two distinct upward trends in *Caspase-3* expression in cardiac muscle between 2 to 24 hours postmortem.
Conclusions: This study innovatively combines NGS with ISH to explore thanatotranscriptomic expression, providing a reference for improved PMI investigation.

54: CHROMOBLASTOMYCOSIS IN A GREAT HORNED OWL (BUBO VIRGINIANUS)

Jessica Lambert[1], Amberly Sokoloff[2], Ji-Hang Yin[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA, [2]Auburn University Raptor Center, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Chromoblastomycosis is one of the most prevalent implantation fungal infections and has been rarely documented in avian species. An adult male Great Horned Owl (*Bubo virginianus*) was presented to the Auburn University Raptor Center at the Auburn University College of Veterinary Medicine with a large, right-sided facial mass. On physical examination, the bird had a poor body condition, dull mentation, pale mucous membranes, and a 7 x 4.5 x 3 cm firm, immobile, pale tan facial mass that surrounded the right eye and extended to the right cere. Due to poor prognosis, the bird was humanely euthanized. Post-mortem and histological examination diagnosed granulomatous dermatitis with numerous intralesional dematiaceous fungi involving the right side of the face, with invasion into the nasal and oral cavities and along the sclera of the right eye. Histopathologic features of the fungi included rare sclerotic bodies, and golden-brown, GMS and Fontana-Masson-positive hyphae that were septate, 2- µm to 6-µm in width, occasionally branching, with up to 25-µm in width terminal bulbous dilations. *Cladosporium* spp. was initially identified morphologically; however, a combination of phenotypic characterization and DNA sequencing targeting at the Internal Transcribed Spacer (ITS) confirmed an infection with *Fonsecaea monophora*. This case report highlights the importance of including chromoblastomycosis as a differential for facial masses in birds and the cruciality of employing a combination of morphology and molecular approaches for accurate fungal identification.

55: OSTEOSARCOMA WITH OSTEOMYELITIS CAUSED BY PSEUDOMONAS SPP AND COELOMITIS IN AN IGUANA IGUANA: CASE REPORT

Sebastián León-Zetina[1], Elisa Maria Garcia-Morales[1], José Juan Diaz-Vertiz[2], Alma Paulina Acosta-Nieves[2], Daniel Rodriguez-Arroyo[3], Angela Maria Mendez-Linares[3], Olivia Lizeth Sanchez-Cervantes[1]
[1]Centro de Diagnóstico Veterinario Bajio, Queretaro, Mexico, [2]Hospital de Especialidades Marisma, Queretaro, Mexico, [3]VETTEM, Ciudad de México, Mexico

A 4-year-old captive patient in good body condition was presented to a private clinical practice for a month's history of forelimb swelling. During the physical examination, the right femoral region was swollen, painful, and firm. Radiographs revealed severe periosteal reaction, concavities, and increased surrounding soft tissue in the mid and distal femur, proximal tibia, and fibula, as well as a

displaced pathological fracture of the lateral femoral condyle. Osteomyelitis and osteosarcoma were suspected.

A fine needle aspiration was performed and submitted for cytological evaluation. Given the cytological findings, it was interpreted as mesenchymal proliferation with multiple cytological criteria of malignancy and suspected osteoid matrix with heterophilic inflammation, so osteosarcoma was considered the main differential diagnosis. Subsequently, the patient presented with ventral pain, and an ultrasound diagnosed coelomitis due to egg rupture. The patient developed dyspnea and was unable to move, leading to the decision to perform euthanasia.

During the necropsy, the coelomic cavity was found to be filled with abundant yellow material of proteinaceous origin (egg rupture). The right femur was enlarged, infiltrating the adjacent bone with multiple cavitations containing necrosis. A bacterial culture of the bone-isolated *Pseudomonas* spp. Histological sections confirmed the diagnosis of osteosarcoma.

There are few reports of osteosarcoma with osteomyelitis in this species, highlighting the relevance of this case description.

56: CEREBRAL AND CEREBELLAR DYSGENESIS WITH NEURONAL HETEROTOPIA AND AGENESIS OF THE CORPUS CALLOSUM IN A DOG

Colleen Lynch[1], Daniel Hicks[2], Allan Pessier[1]
[1]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA, USA, [2]BluePearl Pet Hospital, Tacoma, WA, USA

A 5-month-old male Pharoh Hound was presented for necropsy with a history of intractable seizures and bilateral blindness beginning at 2.5 weeks of age. Postmortem examination revealed multiple structural abnormalities of the brain, including pachygyria, hydrocephalus, agenesis of the corpus callosum, and malformation of the cingulate gyri. Histologically, these gross lesions are associated with disorganization of the cerebral cortical laminae and cerebellar folia in addition to near total absence of the corpus callosum and malformation of the hippocampus and retina bilaterally. Large, dysplastic neurons are distributed throughout the cerebrum with foci of neuronal heterotopia disrupting white matter tracts. Cases of cerebral and cerebellar dysplasia with or without agenesis of the corpus callosum are well described in humans but rarely in veterinary literature. The exception is Dandy-Walker syndrome, with which this case shares some characteristics but lacks the key diagnostic features. Of note, the histologic findings in this case share many similarities with described cases of agenesis of the corpus callosum in humans and mice, which are attributed to developmental abnormalities in neuronal migration and axon development. The specific etiology in this case is uncertain, but its similarity to these described cases provides an interesting example of the comparative pathology across these species and conditions.

57: HISTOLOGIC CHARACTERIZATION OF OPHTHALMIA NEONATORUM IN A 5 DAY OLD DOMESTIC SHORTHAIR KITTEN (FELIS CATUS)

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

HISTORY

A 5-day old domestic shorthair female cat (*Felis catus*) was submitted for postmortem examination after 1-2 days of hyporexia and lethargy. The kitten passed away naturally. All other kittens in the litter died naturally within 7 days of birth.

PATHOLOGIC FINDINGS

Postmortem examination found slight buphthalmia of the right eye and minimal digesta throughout the gastrointestinal tract. Both eyelids were fused and a subpalpebral exudate was not present. The right globe protruded slightly from the orbit, suggestive of true buphthalmia. Histologic examination of the globes found bilateral, moderate, neutrophilic and lymphoplasmacytic anterior uveitis with hypopyon, keratitis and keratic precipitates. Histology of the lungs was consistent with colostrum aspiration, the presumed ultimate cause of death in this kitten.

DISCUSSION

Ophthalmia neonatorum is a relatively common clinical diagnosis in neonatal kittens. Histologic examination of this entity is uncommon, however, since most cases are identified clinically and treated accordingly. Reported causes include Feline herpesvirus-1 (FHV-1), Feline calicivirus (FCV), Chlamydia felis, Staphylococcal/Streptococcal spp., and Mycoplasma spp.. While deep stromal involvement and neutrophils suggest a bacterial component, the lack of purulent subpalpebral exudate and lymphoplasmacytic inflammation could support a primary viral infection. Given the high neonatal mortality, ophthalmia neonatorum may represent an ocular manifestation of fading kitten syndrome in this case. Causes of fading kitten syndrome are numerous, and infectious causes overlap with those of ophthalmia neonatorum. Poor sanitation, in particular, can lead to bacterial ophthalmia neonatorum and sepsis, while insufficient colostrum intake can predispose to FHV-1 and FCV infections. Ancillary testing is pending.

58: COSMETIC AUTOPSY OF ANIMALS: THE CHALLENGE

Ikki Mitsui
Okayama University of Science, Imabari, Japan

Introduction:

Autopsy of deceased animals is crucial for elucidating the cause of death (COD) and mechanism of diseases for the steady improvement of veterinary medicine. The decline in the number of companion animals that are autopsied in Japan is likely due to a negative impression of the conventional autopsy procedure. The use of cosmetic autopsy procedures can mitigate the negative perception of mutilation of the pet.

Materials and Methods:

The author and colleagues performed cosmetic autopsies on approximately 300 animals between 2012 and 2024. While dogs and cats predominated in this cohort, there were also other species such as rabbits, birds, and hamsters. The skin of a dorsally recumbent carcass is incised along the midline from the mandibular to pubic symphysis without hair clipping. The thoracic cavity is opened by sternotomy. The brain is removed in most cases through a u-shaped occipitocervical skin incision and craniotomy. Gross examination, histopathology, and ancillary tests are then performed equivalent to a routine (non-cosmetic) autopsy. All skin incisions are later sutured with clear line, then any blood stains on fur are cleaned by diluted sodium hypochlorite or shampooing. The time spent for a cosmetic autopsy procedure excluding autopsy is 1 to 2 hours depending on the size of the carcass. All the animal owners requested a return of the carcass so that they could then properly dispose of their beloved pets.

Results and Conclusions:

Cosmetic autopsy procedures could reduce the stigma against autopsy for owners who wish to know the COD of their animals.

59: PLASMODIUM CATHEMERIUM AND WEST NILE VIRUS COINFECTION IN A JAVA SPARROW (PADDA ORYZIVORA) DRIVING SURVEILLANCE FOR HEMATOZOA IN THE COHORT AND TRAPPED MOSQUITOES

Erica Moscoso[1], Nicholas Haley[1], Mary White[1], Elaine Corbus[2], Carolina Alvarez[2], Alexis Roth[2], Frank Ramberg[3], Jason Struthers[1]
[1]Midwestern University College of Veterinary Medicine, Glendale, AZ, USA, [2]Reid Park Zoo, Tuscon, AZ, USA, [3]University of Arizona Department of Entomology, Tuscon, AZ, USA

Introduction: Java Sparrows (JASPs) are an endangered finch with population declines due to avian malaria. A 2-month-old, 20 g, captive-bred female was found recumbent with a swollen head and eyes, and later died.

Methods: Postmortem examination, various tissue imprints (TIs) for cytology, and hematozoa PCR followed. Ten mosquitoes were captured nearby. Additionally, samples from 20 live and 6 dead JASPs were submitted for blood film (BF) analysis (2), BF analysis and hematozoa PCR (18), TI analysis (1), and TI analysis and hematozoa PCR (5).

Results: The JASP had fair body condition, hepatosplenomegaly, chemosis, an intact skull, and cerebral hemorrhage with retrobulbar and foramen nervi olfactorii herniation. TIs detected hematozoa and hemozoin. Histopathology detected meronts in the liver, spleen, bone marrow, and lung with inflammation and hemozoin. The brain was acutely disrupted and hemorrhagic, superimposed on chronic vascular reactivity, intravascular hypercellularity, cuffing, gliosis, and encephalomalacia. IHC on brain for West Nile virus (WNV) was immunopositive. Liver PCR detected Plasmodium cathemerium (Pc). For the remaining 26 surveyed JASP, hematozoa were detected by BF and TI cytology (5/26) and PCR (19/23), including Plasmodium relictum (2), and other uncharacterized Plasmodium sp. and Haemoproteus sp. (16). PCR for Pc was negative in all mosquitoes (Culex quinquefasciatus).

Conclusions: Hematozoa were detected in 20/27 (74%) JASPs and involved in the death of the only JASP infected with Pc, although death ultimately was due to head trauma predisposed by chronic-active cerebral lesions caused by WNV. Hematozoa and WNV infect captive JASPs and may compromise species recovery.

60: TRACHEAL OR LARYNGEAL PLASMA CELL TUMORS IN 10 DOGS

Kathleen Mulka, Deborah Gillette, Amy Durham, Elizabeth Mauldin
University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

Background

Canine extramedullary plasma cell tumors (EMP) most commonly arise in the skin, oral cavity, rectum, and colon. Eight case reports describe tracheal and laryngeal plasma cell tumors in dogs, with limited immunohistochemical characterization. While recurrence did not occur in cases of complete excision, follow-up was limited to 3-18 months.

Objective

This retrospective study describes the histopathological and immunohistochemical characteristics and associated clinical signs and outcomes of laryngeal and tracheal EMPs in dogs.

Methods

Five tracheal and five laryngeal EMPs were diagnosed at the Penn Vet Diagnostic Laboratory. Slides were evaluated for histomorphological and immunohistochemical (MUM-1, CD79b, CD20, PAX5, and

CD3) characteristics; one case also had clonality testing (PARR). Clinical information was obtained via submission forms and follow-up questionnaires.

Results

All 10 dogs were male (9 castrated), 7 -15 years old, and of different breeds. Neoplasms were composed of well-differentiated(n=8) to poorly differentiated (n=2) neoplastic plasma cells arranged in sheets, cords, and packets. 3/10 neoplasms were paucicellular with amyloid deposition. All neoplasms labeled positively with MUM-1 and negative for PAX5. There was variable positivity for CD20 and CD79b. All neoplasms were incompletely excised. Follow-up information was available for 7/10 cases. 5/7 cases report no recurrence or clinical respiratory disease at follow up times varying from 6 weeks to 7 years post biopsy.

Conclusions

EMP of the trachea and larynx display heterogeneity in morphology. IHC with MUM-1 and PARR are useful in confirming a diagnosis. Additional follow-up is warranted to further characterize the clinical behavior of EMP at these locations.

61: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL REVIEW OF EASTERN EQUINE ENCEPHALITIS VIRUS MULTI-SPECIES OUTBREAK IN CONNECTICUT, 2023

Arashi Nakashima, Neha Mishra, Emily Reinhardt, Kerr Kirklyn
University of Connecticut, Connecticut Veterinary Medical Diagnostic Laboratory, Storrs, CT, USA

Background: Eastern Equine Encephalitis virus (EEEV), part of the Togaviridae family and Alphavirus genus, is transmitted between mosquitoes and avian reservoir hosts, occasionally affecting incidental hosts like humans and horses. Periodic outbreaks, including one in Connecticut in 2023, highlight its public health significance.

Objective: This study presents the histopathologic and immunohistochemical findings from seven diagnosed cases of EEEV in Connecticut during the reporting year of 2023. The cases include two equines, two ring-necked pheasants, two ravens, and one emu.

Methods: Cases were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) for postmortem examination. Five cases exhibited neurological symptoms and sudden death. Tissue sections from various organs were stained with Hematoxylin and Eosin (H&E). Brain samples were tested for EEEV and West Nile Virus (WNV) via PCR. EEEV immunohistochemistry (IHC) was performed at the Louisiana Animal Disease Diagnostic Laboratory (LADDL).

Results: All cases tested positive for EEEV and negative for WNV by PCR. Histopathologic examination revealed perivascular encephalitis in the horses and pheasants, while the emu and ravens showed neurodegenerative changes with minimal inflammation. IHC revealed strong immunopositivity in neurons and glial cells of the horses and pheasants, whereas the emu and ravens exhibited rare immunopositivity.

Conclusions: This study confirmed EEEV infection in multiple avian and aberrant mammalian hosts. Further this work highlights the comparative neuropathology and immunohistochemical staining seen in different host species.

62: FLUKEY RESPIRATION: PULMONARY TREMATODIASIS AND MIGRATING GRASS AWN IN A 3-YEAR-OLD CANINE

Sai Narayanan, Sarah Myers, Alexandra Ford

College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA

A 3-year-old male intact mixed breed dog from Oklahoma with a history of severe, acute respiratory distress refractory to empirical treatment was submitted to the Oklahoma Animal Disease Diagnostic Laboratory for necropsy evaluation. Clients reported a recent trip to Indiana before acute clinical decline. Significant findings were in the thorax including 2 L of brown-red, opaque, malodorous fluid mixed with numerous, yellow granules. Nearly diffusely, the visceral pleura of all lung lobes was markedly thickened; fibrinous adhesions were frequently between the visceral and parietal pleura and pericardium. The pericardium was diffusely and moderately thickened. Microscopically, the pulmonary interstitium was effaced by severe granulomatous inflammation and too numerous to count, intralesional 25×50 - 50×100 µm, operculated trematode eggs with gold-brown 1-2 µm wide anisotropic shell (interpreted as *Paragonimus sp.).* The pericardium was similarly expanded by granulomatous inflammation surrounding birefringent plant material (grass awn). PCR on affected lung to confirm *Paragonimus kellicotti* infection was inconclusive. Previous studies of *P. kellicotti* indicate a complex life cycle that includes definitive hosts such as domestic and wild canines, felines, raccoons, skunks, red foxes, weasels, humans, etc., and intermediate hosts such as freshwater snails and crayfish. Flukes mature in definitive hosts after ingesting intermediate hosts, migrate to the lungs, and incite granulomatous inflammation. A recently published review lists reports of *Paragonimus* sp., detection in definitive and intermediate hosts in the neighboring states to both Oklahoma and Indiana. These findings indicate *Paragonimus* sp. and migrating grass awns as one of many significant causes for pyothorax in canines.

63: UNCOMMON DOUBLE NEGATIVE (CD4-/CD8-) T CELL LEUKEMIA/LYMPHOMA IN A 3-YEAR-OLD BOSTON TERRIER

Kelly Hubbard, Tyler Harm, Serena Krojanker, Margaret Musser, Ariel Nenninger
Iowa State University College of Veterinary Medicine, Ames, IA, USA

CD4-/CD8-/CD5+ leukemia/lymphoma is an uncommon lymphoid neoplasm described predominantly in young, male, English Bulldogs. It is characterized by morphologically well differentiated small lymphocytes with an aggressive clinical course. Here we report the cytologic, flow cytometric, histopathologic, and immunohistochemical characterization of this uncommon neoplasm in a 3-year-old male castrated Boston Terrier. The patient presented to Iowa State University's Small Internal Medicine Service with a history of hyporexia, vomiting, polydipsia, and icterus. Clinical assessment of the patient identified a mixed hepatopathy, severe thrombocytopenia, and a marked lymphocytosis. Morphologically, the lymphocytes appeared to be small to intermediate in size, and the patient was diagnosed with chronic lymphocytic leukemia (CLL). Flow cytometric analysis was performed and identified this as a CD4- CD8- and CD5+ T-cell lymphoma/leukemia. Due to quality-of-life concerns, the patient was euthanized 5 days after initial diagnosis, and a postmortem examination was performed. Histopathologic examination identified a disseminated round-cell neoplasm affecting the bone marrow, lung, liver, spleen, lymph nodes, kidneys, and intestines, displaying a tropism for the vasculature (angiocentric). The neoplastic round cells appeared to be small to intermediate in size, consistent with the neoplastic lymphocytes identified on the initial blood smear examination. Immunohistochemistry was performed on all tissues and demonstrated the neoplastic lymphocytes to be CD3+ and CD20-. This finding, in combination with the flow cytometry data, confirmed the diagnosis of disseminated T-cell lymphoma/leukemia.

64: PHOTOGRAMMETRY OF GROSS PATHOLOGICAL SPECIMENS: A TIME AND COST-EFFECTIVE TEACHING TOOL IN VETERINARY PATHOLOGY

Samuel Hailes[1], Carlo Bianco[1], Samantha Parkes[1], Ana Ortiz[1,2], Emma Drinkall[1]
[1]School of Veterinary Medicine and Science, University of Nottingham, LE12 5RD, UK, Nottingham, United Kingdom, [2]The Veterinary Pathology Group, Histology, Bristol, United Kingdom

Background

Photogrammetry is an imaging tool that incorporates overlapping 2D images into a 3D structure. Pathology teaching often reliant on physical specimens, with concerns associated with tissue degeneration and safety. With limited time and financial input, photogrammetry can become a resource with multidisciplinary value, and greater accessibility.

Objective

The objective of this study was to detail a methodology for quickly and cheaply generating 3D scans of tissues with a readily available app and conventional tablet computer, to be used for teaching and research purposes.

Methodology

The trial utilised Polycam™ app and platform on two conventional tablet computers. An average of 100 images were taken over 5 to 15 minutes, at multiple angles and heights with minimal additional equipment. The uploading and processing time within the app for each scan took approximately 5 minutes.

Results

The results were mostly clear, detailed, and interactive. Of the fifteen 3D images generated for the trial, several have been used in undergraduate teaching, postgraduate teaching, research, and diagnostic pathology reporting.

Conclusions

In conclusion, with the Polycam™ app, a fast, easy, and cheap methodology has been established for generation of 3D scans for teaching, diagnostics, and research. The next steps involve generating a catalogue of teaching material, and standardising post-processing of 3D images. The students were very engaged and probably this teaching technology favours students and learners with visual, kinaesthetic, and multimodal learning styles. We aim to introduce the 3D images further within teaching, as well as diagnostic and research activities in the Veterinary School.

65: FIBROBLASTIC MENINGIOMA IN AN ALPACA (VICUGNA PACOS) WITH INTERNAL HYDROCEPHALUS

Carlo Bianco[1], Ana Gomez-Vitores[2], Udo Hetzel[3], James Phelan[4], Davide Corbetta[1], Antonia Morey Matamalas[1], Winsome West[1], Alan Wight[5], Ana Ortiz[1,6]
[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham, United Kingdom, [2]Pathology Department, Animal Plant Health Agency, KT15 3 RB, UK, Addlestone, Surrey, United Kingdom, [3]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland, Zurich, Switzerland, [4]Hill & Phelan Equine & Farm Veterinary Surgeons, LN11 8PG, UK, Authorpe, Louth, United Kingdom, [5]Animal Plant Health Agency Veterinary Investigation Centre, Starcross, EX6 8PE, UK, Starcross, United Kingdom, [6]The Veterinary Pathology Group, Histology, Bristol, United Kingdom

Background

The number of pathological submissions of New World camelids (NWCs) to veterinary diagnostic laboratories and academic institutions is rising in the United Kingdom due to the increasing population of NWCs and the heightened veterinary surveillance. Consequently, various conditions, including neoplastic diseases, are being diagnosed more frequently.

Objective

This case describes the macroscopic, histopathological, immunohistochemical, and ultrastructural findings of a meningioma in an 11-year-old male alpaca presenting with non-responsive neurological signs, specifically seizures.

Methods

A comprehensive macroscopic and microscopic post-mortem investigation was conducted, including histopathology, immunohistochemistry, and transmission electron microscopy. Immunohistochemical analysis was performed for pan-cytokeratin, vimentin, glial fibrillary acidic protein (GFAP), and synaptophysin.

Results

A multilobulated mass measuring approximately 4x2x2 cm was identified, originating from the meninges of the tentorium. This mass compressed the mesencephalon and distorted the mesencephalic duct, leading to secondary internal hydrocephalus. Histologically, the mass was composed of spindle-shaped neoplastic cells arranged in short interlacing bundles, separated by varying amounts of collagen, consistent with a fibroblastic subtype of meningioma. Immunohistochemically, neoplastic cells were positive for pan-cytokeratin and vimentin, but negative for GFAP and synaptophysin. Transmission electron microscopy of the neoplastic cells revealed nuclear inclusions and cytoplasmic invaginations, and intermediate filament bundles within the cytoplasm.

Conclusions

This is the first reported case of a meningioma in NWCs. Meningioma should be considered among the differential diagnoses for neurological signs in this species.

Acknowledgements: APHA Miscellaneous and Exotic Farmed Species Expert Group (MEFS); George Alcock for the technical support.

66: DISSEMINATED INTRAVASCULAR LYMPHOMA IN A DOG

Elise Peauroi, Allison Rogich, Paige Albers, Harry Cridge, Cheryl Swenson, Victoria Watson
Michigan State University College of Veterinary Medicine, East Lansing, MI, USA

Intravascular lymphoma is a rare angiotropic large cell lymphoma defined by neoplastic lymphocytes within blood vessel lumina without leukemia or a primary extravascular mass. A 5-year-old female spayed Cavalier King Charles spaniel presented with vomiting and lethargy. Laboratory data revealed mild renal azotemia, proteinuria, regenerative anemia, hyperbilirubinemia, hypoglycemia (persisted with dextrose supplementation), and severe thrombocytopenia. No specific disease process was identified on thoracic radiographs, abdominal ultrasound, or CBC, which lacked lymphocytosis or atypical lymphocytes. The dog was euthanized and necropsy revealed enlargement of the liver, spleen, kidneys, and multiple lymph nodes. The kidneys contained few irregular raised soft tan nodules extending into the cortex. The presumptive diagnosis was round cell neoplasia. Microscopic examination revealed large, pleomorphic, neoplastic, CD3+ lymphocytes within small to medium veins, capillaries, and occasional small arterioles of all organs. Neoplastic cells were predominantly restricted to vascular lumina and thrombi were identified in meningeal vessels; these findings supported a diagnosis of intravascular lymphoma. Antemortem diagnosis of this disease is difficult due to nonspecific clinical signs and a requirement for histopathologic evaluation. Intravascular lymphoma most commonly presents with neurologic signs in dogs, with other clinical signs less

frequently reported. This case presented with predominantly renal, hematologic, and respiratory clinical signs and neoplastic intravascular lymphocytes were found in all organs. Additionally, the WHO classification of intravascular lymphoma in domestic animals mimics the human system, including only intravascular large B-cell lymphoma with no discussion of T-cell or non-T non-B lymphomas, and therefore does not represent the spectrum of canine cases.

67: GRANULOMATOUS HEPATITIS IN A CHACOAN HORNED FROG (CERATOPHRYS CRANWELLI) WITH AMPHIBIAN-TYPE BRUCELLA

Aimee Pepper[1], Shih-Hsuan Hsiao[1], Judilee Marrow[1], Aubrey Engel[1], Martha Delaney[2], Gretchen Anchor[2], Chien-Che Hung[1]
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Zoological Pathology Program, Brookfield, IL, USA

Background: *Brucella* range in pathogenicity in amphibians, with animals displaying subclinical infection, localized skin lesions, or even fulminant systemic disease. The zoonotic potential of amphibian-type *Brucella* is largely unknown, though recent isolation of a *Brucella inopinata*-like strain from an animal keeper with brucellosis suggests the possibility of interspecies transmission. To our knowledge, *Brucella* has only been isolated from one other Chacoan horned frog with bilateral panophthalmitis.

Objective: To characterize a case of granulomatous hepatitis in association with amphibian-type *Brucella* in a Chacoan horned frog submitted for necropsy.

Results: Grossly, the subcutaneous tissues and coelomic cavity exuded abundant yellow, clear, watery fluid (subcutaneous edema and ascites). The liver contained innumerable white, firm, miliary nodules. Histopathology revealed the presence of myriad randomly distributed granulomas with a core of necrotic debris that was further surrounded by epithelioid macrophages, lymphocytes and plasma cells, and rare multinucleated giant cells. Aerobic culture of the liver yielded growth of *Brucella*, with confirmation by RT-PCR (Ct = 22.8). Speciation utilizing Bruce-ladder and Suis-ladder PCR identified atypical *Brucella*. Whole genome sequencing revealed that the isolate clustered in a clonal group with *Brucella* strains identified previously from other amphibians in the United States. GMS and Ziehl-Neelsen staining did not identify concurrent fungal organisms or *Mycobacterium*, and *Mycobacterium* PCR was negative. ISH revealed *Brucella* nucleic acid in association with hepatic granulomas.

Conclusions: To our knowledge, this is the first report of granulomatous hepatitis in association with *Brucella* in a Chacoan horned frog.

68: PRIMARY INTRAOCULAR NEOPLASM OF PRESUMED TRABECULAR MESHWORK ORIGIN IN AN ADULT GERMAN SHEPHERD DOG

Cornelia Peterson, Stefano Pizzirani, Samantha Palermo, Nicholas Robinson
Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, USA

Here we present the clinical and pathologic findings of a tumor arising from the ventrotemporal trabecular meshwork in the left eye of an adult German Shepherd dog. Ocular and systemic diagnostic testing, including ultrasound biomicroscopy, comprehensive clinicopathologic evaluation, thoracic radiographs, echocardiogram, and abdominal ultrasound, failed to identify any evidence for local extension into or metastasis to the trabecular meshwork from a primary neoplasm elsewhere in the animal. Histomorphologic, histochemical, and immunohistochemical characteristics excluded melanocytic, iridociliary epithelial, neuroepithelial, histiocytic, lymphoid, and metastatic epithelial cell origins, rendering a presumed trabecular meshwork origin for this tumor.

69: CLOSTRIDIUM NEONATALE INFECTION IN TWO GROWER PIGS WITH NECROTIZING TYPHLOCOLITIS

Samantha Polk[1], Sophia Ballard[2], Amy Leestemaker-Palmer[3], Andrea Frei[4], Erica McKenzie[4], Pun Sriboonyapirat[1]
[1]Department of Biomedical Sciences, Carlson College of Veterinary Medicine, Oregon State Universtiy, Corvallis, OR, USA, [2]Department of Bacteriology, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, [3]Department of Molecular Studies, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA, [4]Department of Clinical Sciences, Carlson College of Veterinary Medicine, Oregon State University, Corvallis, OR, USA

Clostridium neonatale is most commonly associated with necrotizing typhlocolitis (NEC) in human preterm neonates and neonatal infants in intensive care units. A single publication recorded its detection via PCR from within the ileum of 23 preterm piglets that subsequently developed NEC. This abstract describes the necropsy findings of two 9-week-old Gloucestershire Old Sport gilts that were necropsied following euthanasia for low body condition, hyporexia, diarrhea, and vomiting. Pigs were purchased one week prior within a group of 10 piglets of roughly the same age. Gross examination revealed necrotizing typhlocolitis with severe mucosal edema, necrosis of gut-associated lymphoid tissue, and severe mesocolonic edema. Histologic examination showed necrosuppurative and hemorrhagic typhlocolitis with intralesional bacteria, large ciliated protozoa (*Balantidium coli*), lymphoid depletion and necrosis, submucosal and serosal edema, and vascular thrombosis. Ancillary testing was negative for most common enteric pathogens in swine, including Porcine Rotavirus-A (PRV-A), Porcine Epidemic Diarrhea Virus (PEDV), Swine Delta Coronavirus (SDCoV), Transmissible Gastroenteritis Virus (TEGV), *Lawsonia intracellularis*, and gastrointestinal parasites on fecal flotation. Bacterial cultures of the large intestine, along with 16s sequencing, identified high numbers *Clostridium neonatale* and low numbers *Salmonella enterica* serovar Typhimurium. This report describes two novel cases of acute necrotizing typhlocolitis in two grower pigs attributable to *Clostridium neonatale*. This pathogen should be considered in cases of severe, acute gastrointestinal disease in neonatal, weaning, and grower pigs.

70: CYTOLOGICAL AND HISTOLOGICAL FEATURES OF HIGH-GRADE UNDEFINED GLIOMA IN A DOG: A CASE REPORT

Apisit Pornthummawat[1], Tanit Kasantikul[2], Nlin Arya[1], Sataporn Phochantachinda[1], Piyaporn Eiamcharoen[3]
[1]Mahidol University, Nakhon Pathom, Thailand, [2]Michigan State University, East Lansing, MI, USA, [3]Kasetsart University, Bangkok, Thailand

Background

In 2018, a revised diagnostic classification of canine glioma was proposed by a consortium of veterinary and physician neuropathologists to promote diagnostic consistency and enable comparative studies of canine and human glioma. A high-grade glioma was suspected in an 8-year-old mixed-breed dog by MRI, and the postmortem work-up diagnosis followed the revised classification.

Objective

To characterize the cytological, histological, and immunohistochemical findings of a high-grade undefined glioma classified by the Comparative Brain Tumor Consortium classification.

Results

Modified Wright-Giemsa stained impression smears revealed a highly cellular sample with an eosinophilic fibrillary background. Neoplastic cells were pleomorphic with pale basophilic cytoplasm, indistinct borders, and irregular nuclei. Moderate to marked anisocytosis and anisokaryosis, bizarre large nucleated cells, necrotic debris, and mineralization were observed.

A high-grade undefined glioma was diagnosed. Microscopically, neoplastic cells were arranged in disorganized sheets within neuropil-like stroma. Anaplastic cells had pleomorphic shapes with small to moderate amounts of eosinophilic cytoplasm. Nuclei were polygonal with finely stippled chromatin. Karyomegalic and binucleated cells were observed. Focally, oligodendrocyte-like cells with coarse chromatin were intermingled with atypical neoplastic cells. Microcysts, multifocal mineralization, necrosis, and rows of glomeruloid vasculatures were sporadically seen.

Immunohistochemical staining showed approximately 30% of the neoplastic cells had cytoplasmic processes reacting to GFAP, and less than 10% had nuclear staining with Olig-2. Immunoreactivity for vimentin and PDGFR-alpha was negative.

Conclusion

We presented histological and immunohistochemical findings of a canine high-grade undefined glioma. The impression smears from this case also provided useful information for perioperative cytologic examination in modern veterinary neurosurgery.

71: BLACK OIL SUNFLOWER SEED INGESTION AND SUSPECTED ACUTE LIPID TOXICITY IN 4 ALPACAS

Chelsea Pulter[1], Asli Mete[1], Omar Gonzales-Viera[1], Beckie Perell[1], Emma Deane[2]
[1]California Animal Health and Food Safety Laboratory, UC Davis, Davis, CA, USA, [2]Loomis Basin Equine Medical Center, Penryn, CA, USA

Background: Four adult female alpacas from the same property in Loomis, CA were found to be recumbent, lethargic, anorexic, and had abdominal pain at minimum 2 days after unintended access and ingestion of black oil sunflower seeds.

Objective: To confirm, document and increase awareness of the significant morbidity and mortality associated with a large amount of lipid ingestion by South American Camelids.

Methods: One alpaca died, one was euthanized and necropsied, and two alpacas were symptomatically treated. The necropsied alpaca was found to have ingested numerous black oil sunflower seeds along with erosion and ulceration of the distal esophagus, C1, and C2 chambers. Ancillary tests performed as a general practice were without significant findings.

Results: Three of the four alpacas died or were euthanized, and one survived with outpatient treatment for suspected acute toxicity. No other testing was performed after the large amounts of black oil sunflower seeds and extensive ulcerations were noted in the foregut of the necropsied alpaca and the pearlescent reflux containing few sunflower seed hulls from an alpaca treated in the clinic.

Conclusions: This case series shows significant morbidity and mortality from the ingestion of highly available lipid, in the form of Black oil sunflower seeds. South American Camelid's lipid metabolism is delicate, and we can further confirm that toxic levels of lipid ingestion can be fatal. Care should be taken that high lipid content feed such as black oil sunflower seeds should be kept out of reach and not fed to camelids.

72: AMOEBIC ENTERITIS AND HEPATOCELLULAR NECROSIS IN AN ADULT PANTHER CHAMELEON (FURCIFER PARDALIS) – A CASE REPORT

Alexandra Reddy, Vanessa Oakes, Michelle Todd, Thomas Cecere, Kevin Lahmers, Stephen Smith
Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

An adult, intact-male panther chameleon (*Furcifer pardalis*) was humanely euthanized following a one-week history of bloody urate production, lethargy, and anorexia. Gross necropsy evaluation revealed diffuse intestinal dilatation and transmural dark red-brown to green-tinged discoloration, caseous intestinal contents, and multifocal, pinpoint, white foci throughout all liver lobes. Histopathological analysis of formalin-fixed samples of intestinal and hepatic tissue demonstrated heterophilic and fibrinonecrotic ulcerative enteritis, as well as hepatocellular degeneration and necrosis, with intralesional unicellular trophozoites measuring up to 17 um in diameter consistent with the genus *Entamoeba.* Transmission electron microscopic evaluation of formalin-fixed intestinal samples highlighted ultrastructural features of trophozoites consistent with *Entamoeba*, including pseudopod formation, central nuclei with peripheralized chromatin, and the presence of chromatoid bodies within the cytoplasm. Herein, we report the first recognized case of amoebic enteritis and hepatocellular necrosis in a panther chameleon (*Furcifer pardalis*) to our knowledge. Although one of the most clinically significant diseases of reptiles, pathogenic amoebiasis is rarely reported in lizards, yet it is of critical concern in zoological institutions due to its high mortality in susceptible species such as lizards. Therefore, this case report demonstrates the importance of clinical recognition and histologic diagnosis of amoebiasis in reptile species to enhance their health and welfare under managed care.

73: PNEUMOTHORAX DUE TO EXTENSIVE NECROSUPPURATIVE LARYNGITIS IN A LABORATORY HOUSED YUCATAN MINIPIG

Wencke Reineking, Emily Mocarski, Christopher Morrill, Kathy Heng, Kerriann Casey
Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA, USA

Background

A juvenile female Yucatan minipig was obtained for a surgical enteric study from an external vendor. Following an uncomplicated surgery, the animal exhibited persistent anorexia. Radiographs obtained four days following surgery suggested pneumothorax (left worse than right), and the animal was euthanized.

Results

Grossly, the right laryngeal vocal fold was overlain by fibrin and pus. A 2 x 2 x 7cm peri-laryngeal abscess extended from the thyroid cartilage to the third tracheal ring.

Moderate emphysema was present in the cranial mediastinum, surrounding the cranial aorta and trachea. There was multifocal to diffuse atelectasis of the right and left lung lobes, respectively. The diaphragm was intact, and intrathoracic negative pressure was confirmed. The pulmonary lymph nodes were moderately enlarged.

Histologically, severe chronic necrosuppurative laryngitis was identified. Inflammation extended from the laryngeal lumen, through the laryngeal soft tissues and intercartilaginous spaces, and was continuous with the peri-laryngeal abscess.

Bacteriology of the abscess yielded high numbers of *Escherichia coli* and *Fusobacterium varium*, as well as moderate growth of *Escherichia coli* O157:H7, *Streptococcus suis,* and *Trueperella pyogenes*.

Discussion

The chronicity, location, and mixed bacterial growth of the abscessation are suggestive of penetrating laryngeal trauma prior to arrival at our facility. In human medicine, pneumothorax can be associated with laryngeal fractures and infections. The observed pneumomediastinum and pneumothorax in this minipig were likely exacerbated by intubation and mechanical ventilation during surgery. It remains unclear why negative pressure was partly restored at the time of necropsy; however, reconstitution of negative pressure is a dynamic process.

74: CYTOLOGIC AND HISTOPATHOLOGIC DIAGNOSIS OF WIDE-SPREAD CUTANEOUS NEMATODE (PSEUDOCAPILLAROIDES XENOPI) INFESTATION IN A LABORATORY HOUSED AFRICAN CLAWED FROG (XENOPUS LAEVIS)

Wencke Reineking, Christopher Morrill, Dave K Chu, Kerriann Casey, Flavio Herberg de Alonso
Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA, USA

Background

Female African clawed frogs (*Xenopus laevis)* were housed in 100L tanks and used for oocyte harvest. Decreased oocyte counts and quality were noticed over several months. A single frog presented for white discoloration of the skin, suspected bite wounds on the forelimbs, and epithelial sloughing. Due to lesion severity, the frog was euthanized.

Results

Grossly, the skin was diffusely opaque. On the right forelimb, multiple pinpoint to 0.2cm ulcerations were present.

Impression smears of the cutaneous lesions revealed large sheets of epidermal cells admixed with multiple ~ 2mm long x 50µm wide capillarid nematodes. Uteri occasionally contained several ~ 50µm long bi-operculated oblong embryonated eggs. In addition, numerous oomycete structures were noted.

Histologically, multiple larval and adult nematode cross-sectional profiles were present within the superficial acanthotic and hyperkeratotic epidermis of the back, ventrum, limbs and interdigital webs. Occasionally, intra-uterine bi-operculated embryonated eggs or schistosomes were identified. There was mild multifocal lymphohistiocytic dermatitis.

The nematodes were identified as *Pseudocapillaroides xenopi* by PCR testing of cutaneous swabs.

Discussion

Histologic and cytologic findings of the skin lesions confirmed a cutaneous nematode infestation with secondary oomycete (presumed *Saprolegnia* sp.) infection. *Pseudocapillaroides xenopi* is a common parasite in wild caught and captive clawed frogs. This is the first reported case diagnosed by cytology of a skin scrape. Clinically, the disease is characterized by anorexia and excessive epidermal desquamation with subsequent wasting over several months. Epidermal acanthosis, hyperkeratosis, and intraepidermial nematodiasis can result in morbidity due to impaired respiration, electrolyte homeostasis, and thermoregulation.

75: A RETROSPECTIVE STUDY OF NON-NEOPLASTIC TONGUE LESIONS IN 450 DOGS AND 239 CATS (2010-2020)

Jesse Riker[1], Daniel Rissi[1,2]
[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [2]Athens Veterinary Diagnostic Laboratory, Athens, GA, USA

Background: Tongue biopsy is a common type of surgical biopsy submission at the Athens Veterinary Diagnostic Laboratory (AVDL).

Objectives: To retrospectively describe the type and frequency of non-neoplastic tongue lesions in dogs and cats diagnosed at the AVDL from January 2010 to January 2020.

Methods: Cases were retrospectively searched from the AVDL web-based archive system.

Results: Out of 793 dogs and 408 cats with tongue biopsy submissions during the studied period, 450 (56.7%) and 239 (58.6%) of the lesions were non-neoplastic, respectively. No sex or breed predisposition was evident, except for canine eosinophilic granulomas, which primarily affected Siberian Huskies (30% of eosinophilic granulomas) and Labrador Retrievers (20% of eosinophilic granulomas). Canine non-neoplastic lesions consisted of inflammatory lesions (286 cases, 64% of non-neoplastic lesions) and proliferative lesions (164 cases, 36% of non-neoplastic lesions). Canine inflammatory lesions included ulcerative and/or suppurative glossitis (78%), hyperplastic glossitis of unknown cause (17%), eosinophilic granuloma (3%), and ranula (2%). Canine proliferative lesions included lingual polyps (82%), calcinosis circumscripta (16%), and histiocytic foam cell nodules (2%). Feline non-neoplastic lesions consisted of inflammatory lesions (228 cases, 95% of non-neoplastic lesions) and proliferative lesions (11 cases, 5% of non-neoplastic lesions). Feline inflammatory lesions included eosinophilic granuloma (60%), plasmacytic glossitis (21%), ulcerative and/or suppurative glossitis of unknown cause (17%), and hyperplastic glossitis of unknown cause (2%). Feline proliferative lesions were all lingual polyps (100%).

Conclusions: Although the percentage of non-neoplastic tongue biopsies was roughly the same between species, dogs had increased diversity of diagnoses and increased prevalence of hyperplastic lesions.

76: LYCOPERDONOSIS PNEUMONIA IN A YOUNG DOG

Jessica Rizor[1,2], Jessica Rutten[3], Allison Watson[1]
[1]Veterinary Diagnostic Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA, [2]National Institutes of Health Comparative Biomedical Scientist Training Program, Bethesda, MD, USA, [3]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Lycoperdonosis is a rare respiratory disease that occurs when spores from the puffball mushroom (*Lycoperdon* species) are inhaled. Currently it is unclear if the severe inflammatory response is due to a foreign body response, an allergic reaction, a response to an active fungal infection or a fungal toxin, or a combination of these processes. An 11-week-old female intact Boston Terrier presented to Colorado State University urgent care services for a several days history of respiratory distress and suspected pneumonia. Prior to developing respiratory signs, the dog was observed with an unidentified mushroom in her mouth. She was anesthetized for transtracheal wash and recovered poorly requiring ventilation. At that time humane euthanasia with subsequent postmortem

examination were elected. Transtracheal wash revealed neutrophilic inflammation with no infectious organisms identified. Postmortem examination revealed firm lungs which failed to collapse, with multifocal to coalescing pale gray foci and sank in neutral buffered formalin. The tracheal bronchial lymph nodes were enlarged. Microscopically the alveoli and bronchi were filled with macrophages, rare multinucleated cells, plasma cells, lymphocytes, and fewer neutrophils, erythrocytes, cellular debris, and edema. Randomly distributed, golden-brown, 3-4um diameter, round fungal spores were present both within macrophages and free within the alveolar lumens. Similar inflammation and fungal spores were also within the tracheal bronchial lymph node. Panfungal PCR performed at the University of Florida on formalin fixed paraffin embedded lung matched *Lycoperdon perlatum* (Common Puffball) with 85% identity. Although puffball mushrooms are commonly found in Colorado, reports of Lycoperdonosis in animals and humans are scarce.

77: CUTANEOUS CRYPTOCOCCOSIS IN A HAFLINGER HORSE

Vincenzo Varasano[1], Lorenza Bandera[1], Valentina Petrucci[2], Anastasia Romano[1], Giuseppe Marruchella[1]
[1]University of Teramo, Teramo, Italy, [2]Azienda Sanitaria Locale di Frosinone, Frosinone, Italy

Background: Cryptococcosis is caused by yeast-like fungi, which belong to the *Cryptococcus gattii/ Cryptococcus neoformans* species complex. Equine cryptococcosis is uncommon, it mainly affects the respiratory system and rarely sheds to the skin through the hematogenous route.

Objectives: Reporting the main features of skin lesions caused by *Cryptococcus* spp. in a horse.

Methods: A Haflinger mare developed multiple skin lesions during the last 4 years. Lesions were 1-to-5 cm in size and mostly affected the groin, the pectoral region and the neck. Some lesions were bulging, arranged as clusters, hyperemic, ulcerated, while others were flattened and crusty. The mare was otherwise healthy and showed an optimal body condition score. Considering clinical findings, equine sarcoid was suspected, a couple of large lesions were surgically removed for histopathological investigation and to prepare the autologous vaccine.

Results: Microscopically, a huge number of round or oval yeast-like organisms were seen, 5-10 mm in diameter and typically surrounded by a clear halo. Such microorganisms were arranged as aggregates, thus shaping a characteristic bubble-like appearance, and were occasionally seen in blood vessels. Moreover, they were positive for Grocott methenamine silver stain. Granulomatous reactions with multinucleated giant cells and foci of purulent inflammation were also observed. Considering pathological findings, cutaneous cryptococcosis was diagnosed. The mare is still alive, dozens of skin lesions being still present.

Conclusions: This is one of the few reports of equine cutaneous cryptococcosis, which should be considered in differential diagnosis of neoplastic-like skin lesions in horses.

78: METASTATIC THYROID CARCINOMA WITH ESOPHAGEAL INVASION IN A DOMESTIC SHORTHAIR CAT

Tsukasa Sakashita, Maxime Derré, Charles Artiles, Caroline Fulkerson, Christopher Fulkerson, Camila Mello, Hamideh Esmaeilzadeh, Anushri Srinath, Andrew Woolcock, Tsang Lin
Purdue University, West Lafayette, IN, USA

Background: Thyroid neoplasia is a common cause for hyperthyroidism in cats, but most of these tumors are benign. Thyroid carcinomas are rare in cats, with metastasis of these tumors even rarer.

Objective: To characterize the gross and histopathological features of a thyroid carcinoma with metastasis and invasion in a cat.

Materials and Methods: An adult, male, castrated domestic shorthair cat was presented to Purdue University Veterinary Teaching Hospital with a history of chronic dysphagia and a cervical mass. A malignant cervical neoplasia with pulmonary metastasis was suspected based on clinical examination and imaging. Esophago-gastroscopy revealed a proximal esophageal mural mass causing luminal narrowing. The cat was humanely euthanized due to poor prognosis. Necropsy was performed and gross, histopathologic, and immunohistochemical (IHC) evaluations were pursued.

Results: Grossly, the right thyroid gland was 2.5cm x 1.7cm x 1.5cm, irregularly oval to round, firm, mottled, and white to tan. The adjacent esophagus had roughened mottled raised mucosal surface focally. The lung lobes had multiple small, raised nodules. Microscopically, the right thyroid mass was revealed as thyroid carcinoma with pulmonary metastasis and esophageal invasion, which was further supported by the positive results of IHC staining with thyroglobulin, TTF-1, and PAX8 markers. The esophageal wall was transmurally infiltrated by the neoplastic cells, leading to ulceration.

Conclusions:

To the authors' knowledge, metastatic thyroid carcinoma with esophageal invasion in cats has not been reported previously. The present case demonstrates a metastatic thyroid carcinoma with esophageal invasion in a cat, resulting in esophageal narrowing, ulceration, and dysphagia.

79: LOBULAR DISSECTING HEPATITIS IN A YOUNG LABRADOR RETRIEVER DOG

Kate Sarkan[1], John C. Rowe[2,3], Molly A. Bechtold[2], Christopher Premanandan[1], Megan E. Schreeg[1,3]
[1]The Ohio State University, Department of Veterinary Biosciences, Columbus, OH, USA, [2]The Ohio State University, Department of Veterinary Clinical Sciences, Columbus, OH, USA, [3]The Ohio State University, Comparative Hepatobiliary Intestinal Research Program (CHIRP), Columbus, OH, USA

Lobular dissecting hepatitis is a rare form of canine chronic hepatitis that affects young dogs and has a poor clinical prognosis. A 2.5-year-old, male neutered Labrador Retriever presented for anorexia, melena, and ascites. Abdominal computed tomography revealed an appropriately-sized liver with multifocal, <1- 6mm, hypoattenuating, noncontrast-enhancing nodules and multiple small tortuous vessels medial to the left kidney. Congenital hepatopathy (e.g. congenital hepatic fibrosis) with portal hypertension and acquired portosystemic shunts was prioritized. Following lack of response to supportive care, euthanasia with postmortem examination was elected.

Gross evaluation revealed 2.5 liters of yellow, clear, watery peritoneal fluid and numerous thin tortuous vessels between the left renal artery and portal vein. The liver was diffusely pale red to tan, firm, and had an enhanced reticular pattern. It had a predominantly smooth surface with dozens of multifocal, pinpoint to 5 mm, raised, round, tan nodules extending into the parenchyma on cut section. Histopathology revealed that in most of the parenchyma lobular architecture was disrupted by diffuse fibrous tracts dissecting between individual and clusters of hepatocytes with low numbers of admixed lymphocytes and plasma cells. The remaining tissue was comprised of multifocal nodules of hypertrophied, hypereosinophilic hepatocytes frequently containing lipid (nodular regeneration). Picro sirius red, Masson's trichrome, and reticulin stains highlighted diffuse dissection and disruption of the parenchyma by fine strands of fibrosis, consistent with the diagnosis of lobular dissecting hepatitis.

Lobular dissecting hepatitis should be considered as a differential in young canine patients with hepatopathy, portal hypertension and ascites, and acquired portosystemic shunts.

80: POST-ANESTHETIC MYOPATHY IN HORSES

Carlos Schild[1], Jeff Blea[2], Akinyi Nyaoke[1], Javier Asin[1], Francisco Uzal[1], Eileen Henderson[1]
[1]California Animal Health and Food Safety Laboratory System, University of California-Davis, San Bernardino, CA, USA, [2]California Horse Racing Board, Arcadia, CA, USA

Post-anesthetic myopathy is a rare complication of general anesthesia in horses. In anesthetized horses, the triceps, gluteal, longissimus dorsi, and/or the extensor of the hindlimb muscles may become ischemic. These horses are unable to stand after surgery. This study describes the pathology of two horses with post-anesthetic myopathy.

Necropsy of two horses (3-year-old Thoroughbred and 13-year-old Clydesdale Horse) unable to stand after surgery and euthanized (24-48 hours after surgery) showed pallor/orange discoloration of the triceps, bicep brachii, teres major, and/or psoas, muscles. Myoglobinuria was observed. Microscopically, affected muscles had diffuse monophasic muscle necrosis. Neural and perineural hemorrhage with axonal degeneration in the radial, ulnar, axillary, median, sacral plexus, and/or ischiatic nerves, was observed in both horses. Focally extensive, necrotizing, and pleo-cellular dermatitis with vasculitis and thrombosis, adjacent to the elbow/shoulder region was observed in one horse. Myoglobin casts were observed in the renal tubules of one horse. The cause of hypotension and ischemia with subsequent myonecrosis in horses under anesthesia is unclear. In one of our cases, the lesions in the skin suggest that compression of the skin and muscles occurred while on the surgery table, as in previous studies. Compression of the vena cava by the viscera, due to dorsal recumbency position during surgery, in combination with the hemodynamic effects of the inhalant anesthetics (e.g., hypotension) have also been suggested as a potential cause of ischemia and subsequent polio-myelomalacia in the caudal spinal cord, not observed in our cases. Sub-clinical hypovitaminosis E and selenium deficiency may also be implicated.

81: RECIPROCAL APPARATUS FAILURE DUE TO SUPERFICIAL DIGITAL FLEXOR AND GRASTOCNEMIUS MUSCLES RUPTURE IN A QUARTER HORSE RACEHORSE

Carlos Schild[1], Jeanette Espil[2], Eileen Henderson[1], Francisco Uzal[1]
[1]California Animal Health and Food Safety Laboratory System, University of California-Davis, San Bernardino, CA, USA, [2]Laboratorio de Patología Especial, Universidad Nacional de La Plata, Buenos Aires, Argentina

The reciprocal apparatus (compound of peroneus tertius muscle and the superficial digital flexor muscle [SDFM]), creates flexion of tarsus, stifle, and coxofemoral joints simultaneously. Acute trauma is the most common cause of peroneus tertius muscle, SDFM and/or gastrocnemius muscle (GM) rupture leading to reciprocal apparatus failure (RAF). Coexistent injury of the SDFM and GM results in inability to bear weight on the hock. This study describes a case of RAF in a Quarter Horse racehorse.

Necropsy of a euthanized 2-year-old Quarter Horse colt with history of acute hindlimb lameness during training was performed. Grossly there were complete (both left- and right-hind legs) transverse rupture of the SDFM, and complete (left-hind leg) and incomplete (right-hind leg) transverse rupture of the GM, with severe hemorrhage and edema. These injuries were located at the origin of each muscle near to the supracondylar fossa of the femur. Microscopically, the tendon of both SDFM, in the supracondylar fossa region, had degeneration of the collagen fibers, chondroid metaplasia, and multifocal aberrant regeneration of the tenocytes. The latter lesion was only in the left tendon. The affected muscles had polyphasic myofiber degeneration and necrosis with occasional neutrophils and macrophages mixed with fibrin, edema, and hemorrhage, expanding the endomysium. The evidence of regeneration and chondroid metaplasia in the tendon of the SDFM indicate chronic/previous damage, which, and probably associated with a fast and strong contraction/movement during training, could have contributed to the rupture of the muscles and RAF with loss of the support of the hindlimbs.

82: NINE CASES OF PRESUMED PRIMARY CAUDAL ABDOMINAL HEMANGIOSARCOMA ASSOCIATED WITH THE URINARY BLADDER SEROSA IN DOGS

Maya Schlesinger, Devinn Sinnott, Kevin Woolard
University of California, Davis - School of Veterinary Medicine, Davis, CA, USA

Hemangiosarcoma (HSA) is a commonly diagnosed neoplasm in dogs with important clinical and prognostic implications. HSA arising from the serosa of the urinary bladder and/or soft tissues of the pelvic inlet is poorly described as a primary tumor location and is considered a subset of the overall relatively uncommon retroperitoneal hemangiosarcoma (RPHSA). Nine cases of hemangiosarcoma associated with the urinary bladder serosa or pelvic inlet were identified in necropsy (n=8) and biopsy (n=1) submissions at the University of California, Davis - Veterinary Medical Teaching Hospital (VMTH) between 2003 and 2023. Clinical data and histopathology for these cases were reviewed. Primary presenting complaints included hemoabdomen (n=2), difficulty or changes in urination (n=4), cutaneous bleeding masses (n=1) or other clinical signs unrelated to the abdominal HSA (cerebral disease, n=1; cardiac abnormalities and lethargy, n=1). Patients ranged from 6.3 to 14.1-years-old at the time of diagnosis (mean=10.6-years-old), represented 8 different breeds, and were distributed evenly across sex (male-castrated, n=5; female-spayed, n=4). Grossly, the masses were predominantly dark red to tan, variably cavitated, and associated with hematoma formation. Histologically, these neoplasms were often closely associated with the urinary bladder serosa and neoplastic mesenchymal cells formed cavernous channels filled with hemorrhage and fibrin, characteristic of HSA. These findings demonstrate that the urinary bladder serosa and pelvic inlet are clinically important sites for HSA occurrence and should be considered as a differential diagnosis for dogs presenting with hemorrhagic caudal abdominal masses and urinary dysfunction.

83: SPONTANEOUS ORONASAL EXPULSION OF NEOPLASTIC AND NON-NEOPLASTIC LESIONS IN DOGS

Daniel A. Sebastian Pineda[1], Fabiano N. Oliveira[2], Erin A. Graham[3], Moges W. Woldemeskel[3], Daniel R. Rissi[4]
[1]Department of Pathology, University of Georgia, Athens, GA, USA, [2]Antech Diagnostics, College Station, College Station, TX, USA, [3]Tifton Veterinary Diagnostic and Investigational Laboratory, Department of Pathology, University of Georgia, Athens, GA, USA, [4]Athens Veterinary Diagnostic Laboratory, Department of Pathology, University of Georgia, Athens, GA, USA

Background: Spontaneous expulsion of tissues from body orifices is rare in veterinary medicine.

Objectives: Here we underscore the diagnostic value of tissues spontaneously expelled from the nose or mouth of dogs summited for histologic evaluation at 3 veterinary diagnostic institutions.

Methods: Cases were retrospectively searched (2000–2024) from the Athens Veterinary Diagnostic Laboratory, Tifton Veterinary Diagnostic and Investigational Laboratory, and Antech Diagnostics web-based archive system.

Results: We retrieved 20 submissions. Affected dogs were males (8 castrated and 3 intact) or females (5 spayed and 3 intact) of several breeds; the sex was unspecified in one case. Tissues were expelled from the nose (18 of 20 cases) or mouth (2 of 20 cases). Clinical signs consisted mainly of epistaxis (10 of 20 cases). Neoplastic diagnoses (18 of 20 cases) included sarcoma (8 of 18 cases) or carcinoma/ adenocarcinoma of unknown origin (7 of 18 cases), round cell neoplasm (2 of 18 cases), olfactory neuroblastoma (1 of 18 cases), and adenoma/carcinoma of unknown origin (1 of 18 cases). Non-neoplastic lesions (2 of 20 cases) consisted of fibrinous or suppurative exudate. Autolysis precluded histology in 2 of the neoplasms.

Conclusions: Tissues expelled from the nose or mouth can be suitable for a histologic diagnosis.

84: SPONTANEOUS NEOPLASMS IN NON-COMMERCIAL PIGS: A RETROSPECTIVE STUDY (2010-2024)

Daniel A. Sebastian Pineda[1], Elizabeth W. Howerth[1], Daniel R. Rissi[1], Elizabeth Uhl[1], Xuan H. Teo[1], Brittany Mchale[2], Panchan Sitthicharoenchai[3], Vicente A. Avila Reyes[1]
[1]Department of Pathology, University of Georgia, College of Veterinary Medicine, Athens, GA, USA, [2]Infectious Diseases Laboratory, University of Georgia, College of Veterinary Medicine, Athens, GA, USA, [3]Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Background: Reports of neoplastic disease in commercial pigs are infrequent, most likely because most animals are slaughtered before they reach maturity. Data about neoplasia in non-commercial pigs (i.e., research, pets, and feral) is scarce.

Objectives: To document neoplasms diagnosed in non-commercial pigs at two academic institutions.

Methods: Retrospective database search for cases of spontaneous neoplasms in non-commercial pigs (surgical biopsies and autopsies) from 2010-2024.

Results: We retrieved 56 cases, in which 60 neoplasms were detected. Males were more frequently affected (31/56, 55%) than females (23/56, 41%), with a mean age of 10.2 years. The Vietnamese pot-bellied pig accounted for the most commonly affected breed (55% of total cases). Diagnosed neoplasms consisted of undifferentiated sarcomas (11/60, 18%), adenocarcinomas (9/60, 15%), squamous cell carcinomas (8/60, 13%), melanocytic neoplasms (7/60, 11% of the neoplasms), lymphomas/leukemia (5/60, 8%), hepatocellular carcinomas (4/60, 7%), leiomyomas (3/60, 5%), mast cell tumors (3/60, 5%), interstitial cell tumors (3/60, 5%), adenomas (2/60, 3%), granulosa cell tumors (1 case, 2%), biliary adenocarcinoma (1/60, 2%), pheochromocytoma (1/60 2%), splenic stromal neoplasm (1/60, 2%), and acanthomatous ameloblastoma (1 case, 2%). In 4 cases (%), pigs had more than one neoplasm in different organs.

Conclusions: Carcinomas and sarcomas were the most common neoplasms in our case series. In contrast with recent reports of pig neoplasms, lymphoid neoplasms were not the most common tumors.

85: CUTANEOUS LYMPHOCYTOSIS IN A FENNEC FOX

Kristen Shekelle[1], Alan Glassman[2], Kay A. Backues[2], Jennifer J. Kilburn[2], Desirae A. Wheatcraft[3], Lauren Wood Stranahan[1]
[1]Texas A&M University, College Station, TX, USA, [2]Tulsa Zoo, Tulsa, OK, USA, [3]Oklahoma Veterinary Specialists, Tulsa, OK, USA

A 10-year-old, female, spayed, fennec fox presented for multifocal alopecia and crusting of the axilla, dorsal neck, and lateral thigh of several months. Histopathology of the affected skin revealed diffuse infiltration of the superficial dermis by numerous small, mature lymphocytes, with moderate numbers of eosinophils and fewer mast cells. Scattered lymphocytes multifocally infiltrated the epidermis. The majority of infiltrating lymphocytes expressed cytoplasmic CD3. PCR for antigen receptor rearrangements (PARR) yielded a clonal rearrangement in the T-cell receptor gamma (*TRG*) locus. The histopathologic findings and immunohistochemistry are consistent with cutaneous lymphocytosis. Treatment with oral prednisone resulted in significant improvement of lesions. Cutaneous lymphocytosis is a rare condition in veterinary medicine, with only a small collection of documented reports in cats, and fewer so in dogs. The most common reported cutaneous presentations include multifocal alopecia, erythema, and scale. Histopathology demonstrates a diffuse dermal infiltration of small to medium, CD3 positive lymphocytes with or without mild epitheliotropism. Clonal rearrangement of the T-cell receptor gamma locus is consistent with cutaneous lymphocytosis in

dogs, and as such can be classified as an indolent, small cell lymphoma. The stable progression in this fennec fox case is consistent with the documented disease in dogs and cats. This case report represents the first reported case of cutaneous lymphocytosis in a fennec fox and displays characteristics that represent the features of the condition appreciated in both dogs and cats.

The authors declare that the content of this abstract is based on a forthcoming article.

86: UNCOMMON ACTINOMYCES SP. HYPHAE MORPHOLOGY IN A DOG WITH PLEURITIS AND MEDIASTINITIS

Cierra Starbird, Rachel Neto, Jessica Ball
Auburn University, Auburn, AL, USA

The genus *Actinomyces* is a ubiquitous group of gram-positive, non-acid-fast, facultative to obligate anaerobic, saprophytic, commensal bacteria of many veterinary species and humans. These bacteria are typically pleomorphic, occurring as short rods, curved, or filamentous clusters. In addition, they can exhibit branching resembling fungal organisms microscopically but are typically narrower than true hyphae and lack septations.

A 4-year-old, intact male, Labrador Retriever, dog was presented to the Auburn University Veterinary Teaching Hospital for lethargy, hyporexia, and tachypnea. Approximately 1.5 liters of red-tinged, purulent exudate was removed via thoracocentesis and sampled for aerobic and anaerobic bacterial cultures, yielding light growth of *Actinomyces sp.* A left caudal lung lobectomy and mediastinectomy were performed and the tissue was submitted for histopathology. Histologically, there was a necrosuppurative pleuritis with pulmonary thrombosis and hemorrhage, and the mediastinal tissue contained numerous pyogranulomas centered on mats of radiating, filamentous, Gram-positive, acid fast-negative bacteria intertwined with numerous 3-µm wide, parallel-walled, GMS-positive presumed fungal hyphae, and a polymicrobrial infection was suspected. The histomorphologic and histochemical properties of the filamentous bacteria were consistent with the cultured *Actinomyces sp.,* and paraffin-embedded formalin-fixed tissue was submitted for panfungal PCR, which returned negative for fungal organisms. Given the negative molecular results and literature describing hyphae-like growth of *Actinomyces sp.,* a diagnosis of actinomycosis exhibiting two divergent morphologies is considered most likely. This case demonstrates that *Actinomyces sp.* can mimic fungal organisms in tissue sections which may pose a diagnostic challenge to the pathologist.

87: RENAL ADENOCARCINOMA, CYSTIC CALCULI, AND COELOMITIS IN A FLORIDA SOFTSHELL TURTLE (APALONE FEROX)

Quynn Steichen, Matthew Smith, Heidi Pecoraro
North Dakota State University, Fargo, ND, USA

An aged adult, male Florida softshell turtle (*Apalone ferox*) was examined after being found acutely dead in its enclosure with no previous clinical signs. Bilaterally, the kidneys were enlarged and had multiple, round, pale-yellow, bulging masses with friable, gritty material on section. Histopathology of the kidneys revealed effacement by a non-encapsulated, poorly demarcated, tubular, and papillary mass with cystic, flocculent, eosinophilic material. The gross and histopathologic findings support the diagnosis of renal adenocarcinoma.

Additionally, *Aeromonas hydrophilia* and *Elizabethkingia miricola* were isolated from the coelomic cavity. These are bacterial pathogens commonly cultured in reptiles and amphibians and reported to cause dermatitis, pneumonia, osteomyelitis, and septicemia. In the bladder were three cystic calculi composed of predominately calcium phosphate carbonate.

Renal adenocarcinomas are most commonly reported in snakes and lizards and are rarely mentioned in chelonians. The histopathologic features in this case parallel other case reports of renal adenocarcinomas in snakes. However, in this case, there is bilateral involvement of the kidneys and no evidence of metastasis. To aid in the diagnosis of these tumors in domestic animals and snakes, immunohistochemistry (**IHC**) may be beneficial. To our knowledge, there are no reports of using IHC to diagnosis renal adenocarcinomas in chelonians. Further investigation is warranted to increase the level of confidence of this diagnosis in non-domestic species.

88: CORNEAL STROMAL MESENCHYMAL TUMOR IN A DOG

Aarathi Sureshkumar, Brittany Rasche
Kansas State University, Manhattan, KS, USA

A 9-year-old neutered male mixed breed dog underwent enucleation of the right eye due to corneal thickening and opacity. When sectioned, the cornea was markedly expanded by a firm pale tan mass.

Histologic examination of the eye revealed a poorly demarcated, non-encapsulated neoplasm occupying 80-90% of the corneal stroma and consisting of spindle cells arranged in densely cellular interweaving streams, occasionally with a herringbone pattern, supported by minimal fibrous stroma. The neoplastic cells did not extend into the overlying corneal epithelium or other parts of the eye. Intermixed with the neoplastic cells were many plasma cells, lymphocytes, and Mott cells as well as several small caliber blood vessels.

Immunohistochemical staining revealed positive immunolabeling of the neoplastic cells for vimentin and S100 and lack of immunolabeling for pancytokeratin, smooth muscle actin (SMA), PNL2, Melan A, GFAP, and Olig2. These findings confirmed mesenchymal differentiation of this neoplasm and may suggest nerve sheath origin given positive S100 immunolabeling. However, S100 is not specific for nerve sheath tumors. GFAP and Olig2 immunoreactivity are reported to have variable expression in nerve sheath tumors but were absent in this case. Lack of Melan A, PNL2, and SMA immunoreactivity makes a diagnosis of melanoma or perivascular wall tumor less likely.

Corneal stromal mesenchymal tumors are exceedingly rare in dogs with, to our knowledge, only a single published case report of a dog with bilateral corneal nerve sheath tumors. Thus, this may represent only the second reported case of corneal nerve sheath tumor in a dog.

89: COLONIC INTRAMURAL PANCREATIC PSEUDOCYST IN A DOG: A CASE REPORT

Parvathy Thampi[1], David Pendray[2], Paula Schaffer[1]
[1]College of Veterinary Medicine and Biological Sciences, Department of Microbiology, Immunology & Pathology, Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO, USA,
[2]Advanced Veterinary Care Center, Cody, WY, USA

A 9-year-old male castrated English Setter/Labrador mix presented to a referral practice with intermittent vomiting, hypoglycemia, and elevated insulin/glucose ratio for a presumed insulinoma. Exploratory surgery revealed a pancreatic mass, which was histologically diagnosed as neuroendocrine carcinoma. Approximately 4 days later, the dog presented with abdominal pain, hyporrhexia with neutrophilia, and positive canine pancreatic lipase. Inpatient supportive care for pancreatitis was instituted, and the patient was discharged 12 days postop. Ten days later, the patient had a hunched posture and was straining to defecate. Abdominal ultrasound 37 days postop revealed a midabdominal cystic structure. Exploratory laparotomy confirmed a periduodenal cystic lesion and a separate thick-walled cystic lesion along the transverse colon. Fluid cytology and lipase levels of the periduodenal cystic lesion were consistent with a pancreatic pseudocyst. While attempting to isolate the colonic lesion, the dog suffered acute cardio-respiratory arrest. The colonic

lesion was resected and submitted for histopathological examination. On gross and microscopic examination, the pancreas was adherent to the colon and a large pseudocyst dissected through the colonic muscularis and impinged upon the colonic lumen. This cavity was bordered by approximately 1-2 mm of granulation tissue and contained abundant fibrin, hemorrhage, neutrophils, and cell debris. The adjacent pancreatic acini were compressed by dense bands of connective tissue (pancreatic phlegmon). To our knowledge, this is the first report of pancreatic pseudocyst forming in the colon of a dog. This case report aims to raise awareness and contribute to the limited veterinary literature on pancreatic pseudocysts in dogs.

90: MICROSCOPIC FINDINGS ASSOCIATED WITH MULTIPLE MICROBIAL INFECTIONS IN A COLONY OF BUMBLEBEES (BOMBUS IMPATIENS)

Luis Tondo[1], William Holl[1], Naomi Falconnier[1], Adrian Izquierdo[2], Cesar Menk[1], Mariano Carossino[1]
[1]Lousiana State University, Baton Rouge, LA, USA, [2]Audubon Nature Institute, New Orleans, LA, USA

Background: Bumblebees (*Bombus impatiens*), a common eastern North American eusocial bee, have been suffering population decline due to environmental influences, diseases, and non-native species.

Objective: The aim was to describe microscopic features of multiple microbial infections in deceased and euthanized bumblebees.

Methods: Increased mortality was reported in a captive bumblebee colony. Four naturally deceased and 18 euthanized bumblebees were subjected to microscopic examination and brood health surveillance.

Results: Fungal hyphae, with variably associated inflammation and melanization, were observed in euthanized (8) and naturally deceased (1) bumblebees, affecting the cuticle and epidermis, hemocoelom, chitinous and mid-gut, and Malpighian tubules. Melanization and tissue necrosis, with intralesional cocci bacteria, was observed in the cuticle and epidermis in deceased bees (3). Trypanosomatids were adhered to the ileal epithelium of 15 bees, with frequent concurrent fat body atrophy. External mites were observed in 5 euthanized bees, and intraepithelial protozoans were within the midgut of deceased bees (2). A mite collected from the colony was identified as *Parasitellus* sp.

Conclusions: Fungal and bacterial infections with associated melanization were frequent. Trypanosomatids were identified in all bees and likely represent *Crithidia bombi,* which has been associated with wasting disease in bumblebees and decrease in colony fitness. Microscopically observed mites represent the identified *Parasitellus* sp., which are detritivores; and the intraepithelial protozoan are of undetermined clinical significance. These changes and organisms may reflect unfavorable husbandry conditions, predisposing to polymicrobial infections and disease in the colony. Further research is needed to better characterize infectious agents in bumblebees.

91: NON-INFECTIOUS CHRONIC AMNIONITIS AND AMNION NODOSUM LATE ABORTION IN AN EQUINE FETUS

Luis Tondo, Cesar Menk, Fabio Del Piero
Lousiana State University, Baton Rouge, LA, USA

Background: The fetal hair can cause chronic amnionitis due to friction against fetal membranes producing nodularities referred as *amnion nodosum* in horses and humans, and can be associated with abortion.

Objective: To report a case of chronic nodular amnionitis (*amnion nodosum*) as a cause of a late abortion in an equine fetus.

Methods: An 11-month-old aborted equine fetus with all placental membranes was submitted for postmortem evaluation. Routine gross and histologic examination were performed for all samples.

Results: The amnion was diffusely thickened with multiple irregular yellowish nodules and plaques, and markedly tortuous and thick-walled blood vessels. Microscopically, the plaques corresponded to necrosis, squamous metaplasia, and granulation tissue, with embedded hair shaft fragments. In the amnion, multiple nodular spaces were acellular and filled with amorphous eosinophilic material and cellular debris. Underneath the chorioallantois allantoic epithelium there were areas of collagenous thickening.

Conclusions: The changes in the placenta, especially the nodular structures in the amnion are consistent with *amnion nodosum*. The described changes, likely due to mechanical friction which could have been predisposed by oligohydramnios and the large size of the fetus, justified the mare's late abortion.

92: COMBINED STRONGYLUS EDENTATUS AND STRONGYLUS VULGARIS PARASITISM IN A WARMBLOOD FOAL IN ONTARIO, CANADA

Andrii Trokoz, Emma Borkowski
University of Guelph, Guelph, ON, Canada

A 6 month old Warmblood filly presented for acute profuse diarrhea unresponsive to treatment and severe wasting was euthanized after progression to obtundation. Gross examination revealed multifocal hemorrhage within the extraperitoneal fascia and diaphragm with intralesional 1.5 cm white nematodes. Additionally, verminous arteritis of the cranial mesenteric artery, colonic artery thrombosis with segmental colonic infarction, ulcerative colitis, and segmental fibrinonecrotizing enteritis were present. Parasitological and molecular evaluation of nematodes from the extraperitoneal fascia and diaphragm confirmed *Strongylus edentatus* infection. In turn, histopathology identified intralesional nematodes within the thrombosed cranial mesenteric artery, consistent with *Strongylus vulgaris*. Bacterial culture of the colon and small intestine confirmed *Salmonella* spp. and *Lawsonia intracellularis*, respectively. *S. edentatus* larvae are much larger than *S. vulgaris* and undergo extensive fascial migration producing hemorrhage, but are very rarely diagnosed in North America. In this case, it is thought chronic bacterial enteritis contributed to immunosuppression and unusually high larval strongyle burdens.

93: MULTIPLE ENDOLARYNGAL, ENDOTRACHEAL & ENDOBRONCHIAL CHONDROMA WITH CONCENTRIC BIVENTRICULAR CARDIAC HYPERTROPHY, SECONDARY EDEMA & ALVEOLAR EMPHYSEMA

Juan Pablo Velasco Montes de Oca[1], Armando Javier Villarreal Sánchez[2], Miguel Rodríguez Murillo[1]
[1]Diagnóstioc Veterinario del Valle, CDMX, Mexico, [2]Clínica Vewterinaria French's, Torreón, Mexico

A 6-year-old chow chow dog presented with respiratory difficulty, dysphonia and signs of respiratory stenosis. Endoscopy revealed multiple nodules in the laryngeal, tracheal and main bronchi lumen measuring up to 1.2 cm. Euthanasia was elected and necropsy was performed. Those lesions microscopically correlated to a matrix of hyaline cartilage containing chondrocytes and chondroblasts, with slight anisocytosis, without mitotic activity, that were non-infiltrative, and were confined to the subepithelial compartment. Additionally, alveolar emphysema, hypertrophy of the muscular-vascular tunic, and changes associated with chronic expectoration were found in the lungs. The heart exhibited concentric biventricular hypertrophy.

These findings characterize neoplasia of chondroid histogenesis, with multifocal distribution, and of primary origin. These tumors could have led to the development of secondary pulmonary

hypertension and cardiac hypertrophy. The presentation of this neoplasms in both animals and humans is infrequent, mainly affecting the phalanges in people, while in canines it more frequently affects flat bones and sinus osteocartilage.

In canines, tumors of the larynx, trachea and bronchi represent less than 1% of primary neoplasms, the majority are malignant and of epithelial origin, the most common being squamous cell carcinoma and adenocarcinoma. Those with cartilaginous differentiation include chondrosarcoma, osteochondroma, chondroma and myxochondroma.

Some similar entities are laryngeal osteochondroma, with no reports of multifocal distribution, and synovial chondromatosis, a non-neoplastic entity, in which intra-articular projections occur, formed by mature hyaline cartilage. To the best of the author's knowledge, no reports of multiple chondromas with laryngeal-tracheal-bronchial involvement, have been described in canines to date.

94: UTILIZING A LARGE LANGUAGE MODEL FOR INFORMATION EXTRACTION FROM VETERINARY ELECTRONIC HEALTH RECORDS

Judit M Wulcan[1], Jonatan Wulcan[2], Kevin L Jacques[1], Mary Ann Lee[3], Samantha L Kovacs[1], Nicole Dausend[4], Lauren E Prince[4], Sina Marsilio[4], Stefan M Keller[1]
[1]University of California Davis, School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology, Davis, CA, USA, [2]Independent researcher, Malmoe, Sweden, [3]Colorado State University, College of Veterinary Medicine and Biomedical Sciences, James L. Voss Veterinary Teaching Hospital, Fort Collins, CO, USA, [4]University of California Davis, School of Veterinary Medicine, Department of Medicine and Epidemiology, Davis, CA, USA

Background: The lack of efficient methods for extracting information from free text in electronic health records (EHRs) poses a barrier to conducting large retrospective studies in veterinary pathology. Manual extraction is time-consuming, subjective, and error prone. Large language models (LLMs) can interpret and summarize free text, but their accuracy for information extraction from veterinary EHRs is uncertain.

Objective: This study evaluates the performance of a LLM in extracting information from veterinary EHRs compared to humans.

Methods: Using GPT4o (Open AI, San Francisco, USA) at temperature 0 and five humans, the presence of six clinical signs related to feline chronic enteropathy (FCE) was extracted from 250 randomly selected EHRs from the Veterinary Medical Teaching Hospital at the University of California, Davis.

Results: The LLM's performance compared to the majority opinion of the five humans, averaged across all six clinical signs, was as follows: Sensitivity: 97% (interquartile range [IQR] 93-99%), specificity: 98% (IQR 97-99%), positive predictive value: 81% (IQR 71-85%), negative predictive value: 100% (IQR 99-100%), F1 score: 84% (IQR 77-90%) and balanced accuracy: 96% (IQR 95-98%). Repeated LLM runs were more concordant than human assessor pairs (average Cohen's kappa 98 % (IQR 98-99 %) vs. 80% (IQR 78-81%), respectively). Most errors occurred in questions with human disagreement (35/43 [81%]).

Conclusion: Automated information extraction from veterinary EHRs using an LLM is a viable alternative to manual extraction by humans. This approach could enable more efficient, scalable, and objective analyses of datasets, facilitating integration of clinical data with pathology findings in retrospective studies.

95: POST-VACCINAL DISTEMPER ENCEPHALITIS IN A DOG

Heather Yee, Leyi Wang, Jonathan Samuelson
University of Illinois at Urbana-Champaign, Urbana, IL, USA

A 5-month-old, intact male, Rottweiler dog presented for acute onset of neurological signs, including seizures, facial twitching, hyperesthesia, hypersalivation, circling and confusion. MRI revealed multifocal encephalitis. Seizures could not be managed with anti-seizure medications, and humane euthanasia and necropsy were elected. Grossly, the brain was unremarkable. Microscopically, the brain had multifocal lymphohistiocytic encephalitis with neuronal necrosis, gliosis, and intranuclear and intracytoplasmic inclusion bodies. The cervical spinal cord had a focus of mineralization. Grossly, the myocardium at the apex of heart contained multifocal white streaks that were identified as multifocal to coalescing mineralization on histopathology. Fluorescent antibody examination of the brain for rabies was negative. Real-time polymerase chain reaction of the brain was positive for Canine Distemper Virus (CDV) and negative for *Neospora caninum* and *Toxoplasma gondii*. Sequencing of the brain indicated that the CDV strain in the dog is highly related to a strain found in a vaccine. Mineralization in the heart and spinal cord are suspected to be indirect secondary changes from CDV. The dog was reported to be vaccinated against CDV. Given this history and the sequencing results, this is a classical case of post-vaccinal distemper encephalitis.

Clinical Pathology Posters

1: CYTOLOGIC, HISTOLOGIC, AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF A FIBROSARCOMA WITH KELOIDAL FEATURES IN A RHODESIAN RIDGEBACK

Basant Ahmed, Russell Sakacs, Allyssa Cole, Megan Schreeg, Hamideh Esmaeilzadeh
Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

An 11-year-old Rhodesian Ridgeback presented for excision of a 12-cm-diameter ulcerated subcutaneous mass on the ventral abdomen. Fine needle aspirates were collected, and cytology revealed aggregates of round to fusiform cells that were admixed with variably sized but mostly thick bundles of hyalinized bright eosinophilic collagen. Cells had wispy margins with scant to moderate pale blue cytoplasm. Nuclei were round to oval with coarse chromatin, one to multiple prominent nucleoli, and moderate anisocytosis and anisokaryosis. Surgical excision followed by histopathologic examination revealed a poorly demarcated subcutaneous neoplasm composed of fusiform to round cells arranged in short streams supported by collagenous stroma, which was occasionally arranged in well-defined, homogenous, brightly eosinophilic beams, suggestive of keloidal collagen. Anisocytosis and anisokaryosis were moderate, and mitoses were rare. The assigned morphologic diagnosis was a fibrosarcoma with keloidal features (grade I). Neoplastic cells were vimentin immuno-positive, consistent with mesenchymal cell origin, and smooth muscle actin and CD18 immuno-negative, ruling out leiomyosarcoma and round cell tumors, respectively. Mast cell tumor was ruled out by negative immunolabeling for c-Kit, Toluidine blue, and Giemsa histochemical stains. The presence of hyalinized collagen fibers is a distinctive cytologic feature of keloidal fibrous tumors, but histopathologic examination is required to differentiate benign from malignant tumors. Similar collagen can also be present as supporting stroma in mast cell tumors. In this case, this differential diagnosis was excluded via histochemical and immunohistochemical staining. This case characterizes the cytologic, histologic, histochemical, and immunohistochemical presentation of fibrosarcoma with keloidal features.

2: QUALITATIVE AND QUANTITATIVE EVALUATION OF A DRAWING GAME DESIGNED TO DEVELOP VISUAL OBSERVATIONAL SKILLS IN VETERINARY STUDENTS LEARNING CYTOLOGY

Amber Allain, Melissa Meachem, Ryan Dickinson, Nicole Fernandez
Western College of Veterinary Medicine, Saskatoon, SK, Canada

Many areas of veterinary medicine demand accurate, unbiased observation, including diagnostic microscopy. Fine arts-based training and drawing exercises have been used to develop medical and veterinary students' visual observational skills and improve learning retention. In this study, we created an in-class drawing game in which students took turns describing a microscopic image for their partner to draw, with the aim of reinforcing visual and descriptive skills in veterinary students learning cytology. The study used a pre-/midpoint-/post-test design, with students completing two rounds of drawing, then swapping roles between "describer" and "drawer" and completing two more rounds of drawing. Tests were evaluated qualitatively as well as scored using an expert rubric. Scores were compared between pre- and post-tests to evaluate overall effect of the game on diagnostic accuracy, and between pre- and midpoint-tests of describers *versus* drawers to evaluate the effect of role. Students also completed a questionnaire on their perceptions of the drawing game. There was no significant difference between pre- and post-tests or between roles, but questionnaire responses indicated students enjoyed the game and found subjective benefit, including an increased understanding of the importance of observational and descriptive skills. Student descriptions highlighted weaknesses in identifying cell features such as cytoplasmic borders and distinction between cytoplasm and nucleus, which may indicate areas to target in cytology education. Overall, our results indicate that the drawing game provided qualitative benefits and promoted student engagement, and could be adapted for use in other visual subjects, such as radiology or gross pathology.

3: B-CELL MALIGNANCY IN TWO HORSES WITH MONOCLONAL GAMMOPATHY AND PRESUMPTIVE DEVELOPMENTAL TRANSITION

Camila Benaduce Emanuelli Mello[1], Andrea Pires dos Santos[1], Craig A. Thompson[1], Tsang-Long Lin[1], Maria Buman Ruiz Diaz[1], Camilla A. Jamieson[1], A Russel Moore[2], Julia B. Felippe[3], Joanne B. Messick[1]
[1]Purdue University, West Lafayette, IN, USA, [2]Colorado State University, Fort Collins, CO, USA, [3]Cornell University, Ithaca, NY, USA

Background: Multiple myeloma oncogene-1(MUM-1) is not commonly used in horses for a diagnosis of B-cell malignancy with no suspicion of plasma cell tumor.

Cases Description: an 18-year-old, castrated Haflinger equine (case 1; ASVCP 2023) and a 15-year-old Paint mare (case 2) were presented to Purdue University due to multiple skin masses and a single mass on right flank, respectively. Complete blood counts (CBCs) showed leukocytosis and a predominance of lymphocytes with cerebriform nuclei. PCR for antigen receptor rearrangement (PARR) assay on blood and skin neoplasm (case 1) and on peripheral blood (case 2) revealed a clonally rearranged immunoglobulin gene with a monoclonal gammopathy identified by serum protein electrophoresis. Leukemic cells had down-regulated expression of cell surface B-cell markers by Flow Cytometry (case 2). Masses involving multiple organs and lymphadenomegaly was identified on necropsy. Histopathology and immunohistochemistry (IHC; CD3, CD20 and Pax-5) were interpreted as T-cell rich B-cell lymphoma (TCRBCL, #1) and B-cell lymphoma (#2). In addition, approximately 35% (case 1) and 10% to 20% (case 2) of neoplastic cells showed MUM-1 nuclear immunoreactivity, suggesting a developmental transition between germinal center B-cell and plasma cell stages. MUM-1+ neoplastic cells have been associated with a poor prognosis in cases of diffuse large B-cell lymphoma (DLBCL) in humans. MUM-1+ neoplastic cells were also recently described in a few cases of canine DLBCL. Therefore, including this marker in the IHC panel to identify the specific lineage and

developmental stage of lymphoma and/or serve as a prognostic indicator in veterinary species deserves further attention.

4: PRESUMPTIVE PSEUDORETICULOCYTOSIS BY THE ADVIA 2120 IN TWO BOVINES WITH AN EMERGING TICK-BORNE PROTOZOAL DISEASE, THEILERIA ORIENTALIS

Alexandra Bonney[1], Russell Sakacs[1], Laurie Millward[1], Joanne Messick[2], Hamideh Esmaeilzadeh[1]
[1]Department of Veterinary Biosciences, The Ohio State University, College of Veterinary Medicine, Columbus, OH, USA, [2]Department of Comparative Pathobiology, Purdue University, College of Veterinary Medicine, West Lafayette, IN, USA

A two-year-old Shorthorn heifer (Patient One) presented with persistent vaginal bleeding and pale mucus membranes. Her CBC showed a severe, regenerative anemia (HCT 11%, RI: 22 – 37%), marked reticulocytosis (270.2 $x10^9$ cells/L, RI: 0.0 – 0.0 $x10^9$ cells/L), moderate thrombocytopenia (54 $x10^9$/L, RI: 152-594 $x10^9$ cells/L), and mild monocytosis (0.3 $x10^9$ cells/L, RI: 0.00-0.10 $x10^9$ cells/L). Review of the blood smear revealed occasional erythrocytes with one to two small, linear to signet ring-shaped, basophilic inclusions, most consistent with protozoal piroplasm. Also, rare lymphocytes with small, round, eosinophilic granules most consistent with "Koch's blue bodies" (schizonts) were identified. A three-year-old mixed breed beef cow (Patient Two) from the same farm accompanied Patient One as a potential blood donor. Patient Two's CBC revealed a severe, regenerative anemia (HCT 15%), mild reticulocytosis (46.7 $x10^9$ cells/L), and similar erythrocyte inclusions. Whole blood duplex real-time polymerase chain reaction assay was consistent with *Theileria orientalis*, an emerging tick-borne protozoal disease in the United States. The marked reticulocytosis (Patient One), low to moderate numbers of polychromatophilic cells on the blood smear, and atypical reticulocyte scatter plots (ADVIA 2120 analyzer), showing a less-tapered comet tail pattern with more reticulocyte events concentrated in the high fluorescence region (Patient's One and Two), supported a presumptive diagnosis of pseudoreticulocytosis. Manual reticulocyte counts using new methylene blue stain were not performed, but could have confirmed that the analyzer reported erroneously high reticulocyte counts. To the authors' knowledge, presumptive pseudoreticulocytosis by the ADVIA 2120 has not been reported in bovine species.

5: DYSMORPHIC ERYTHROCYTES IN THE URINE OF TWO DOGS AND ONE CAT WITH CONFIRMED AND/OR SUSPECTED GLOMERULOPATHY AND HEMATURIA

Tahj Boston[1], Jessica Hokamp[1], Dennis Chew[2], Mary Nabity[1]
[1]Texas A&M University, College Station, TX, USA, [2]The Ohio State University, Columbus, OH, USA

Background: Dysmorphic erythrocytes describe an abnormal erythrocyte morphology in urine that has been reported in people with glomerulopathies due to passage through damaged glomerular basement membranes. Microscopic evaluation for dysmorphic erythrocytes in routine urinalysis can potentially be helpful in veterinary medicine to identify the origin of hematuria.

Objective: This study sought to introduce and describe the morphology of dysmorphic erythrocytes in urine from dogs and cats with confirmed or suspected glomerulopathy.

Methods: Urine samples from a dog with X-linked hereditary nephropathy (XLHN) and those submitted to the International Veterinary Renal Pathology Service were screened for dysmorphic erythrocytes during sediment evaluation. Selected sediments were placed in Trump-McDowell's fixative for scanning electron microscopy (SEM).

Results: Cases included a 7-year-old male castrated Labradoodle (case 1), 11-year-old female spayed Ragdoll cat (case 2), and 8-month-old male intact mixed breed dog (case 3). Case 1 had proteinuria and hypoalbuminemia secondary to biopsy-confirmed immune complex-mediated glomerulonephritis. Case 2 had macroscopic hematuria suspected to be secondary to glomerular

disease based on the presence of abnormal urine erythrocyte morphology that differed from peripheral blood morphology and lack of lower urinary tract signs. Renal biopsy was not performed. Case 3 had microscopic hematuria and progressive renal disease due to XLHN. Dysmorphic erythrocytes had 1 or more vesicle-shaped protrusions on light microscopy; SEM results are pending.

Conclusions: Glomerulopathy was confirmed or strongly suspected in all three cases with dysmorphic erythrocytes in urine. Although an uncommon feature, identification of dysmorphic erythrocytes during routine urinalysis could strengthen suspicion for glomerular disease.

6: PILOT STUDY TO EVALUATE NOVEL METHOD FOR EXTRACTION OF INTERSTITIAL FLUID FROM HORSES

Bonnie Bragdon[1], Randolph Stewart[1], Christoph Schroeder[2], Ronny Schmidt[2]
[1]Kiffik Animal Health, Providence, RI, USA, [2]Sciomics GmbH, Neckargemünd, Germany

Background: Interstitial fluid (ISF) is an ideal fluid for analysis. ISF requires no processing and is rich in known and novel biomarkers. Current ISF extraction methods cause inflammation or reduce sensitivity due to dilution. A new method for collection has been developed which uses electroporation to create micropores for continuous collection.

Hypothesis: Electroporation allows extraction of quality ISF to allow protein microarray analysis.

Animals: Two healthy, adult horses.

Methods: Pilot study. Horses were fitted with a 16 micropore extraction device. Electroporation was performed for 15 minutes, after which negative pressure was applied. Extraction continued for 23 hours. Horses were confined to individual stalls for 22.5 hours during collection, followed by 0.5-hour free choice exercise. Blood collection was timed with ISF collection. All samples were deep frozen, and scioDiscover protein microarray analysis performed.

Results: After electroporation, ISF was successfully collected at 18, 23, and 23.5 hours from one horse and at 17 hours from the other. An adequate volume of ISF was collected during 30 minutes of exercise to allow protein microarray analysis. Proteins analyzed totaled 1,438. ISF bulk protein concentration ranged from 38.9 to 53.9 mg/mL. Differences in protein abundance were measured between horses, between sample type, and between activity levels.

Conclusions and Clinical Importance: Electroporation is effective for collection of quality ISF in horses to allow for protein microarray analysis and differentiation of proteins based on individual age and activity. Distinct proteins were identified and measured in ISF in concentrations that differed from those in blood.

7: UNEXPECTED OUTCOME OF A SYMPTOMATIC CASE OF SPLENIC MYELOLIPOMA IN A CAT

Helena Ferreira[1], Melanie Dobromylskyj[2]
[1]Zoetis Virtual Laboratory, Marldon, United Kingdom, [2]Finn Pathologists, Harleston, United Kingdom

A 13-year-old female neutered Domestic Shorthair cat presented with anorexia and a mobile, 6cm diameter abdominal mass detectable on physical examination and by radiography, although the organ of origin could not be determined. A fine needle aspirate from the mass revealed high nucleated cellularity on a basophilic background with large numbers of lipid globules, moderate numbers of red blood cells and spicule-like structures with black-staining material resembling iron stores. The nucleated cells comprised small to large lymphoid cells and hematopoietic cells (80% late erythroid precursors, 10% late granulocytic precursors, 6% early granulocytic precursors and 4%

early erythroid precursors). Rare megakaryocytes, binucleated cells, giant band neutrophils and normal mitotic figures were seen. The cytologic findings were interpreted as representative of a splenic myelolipoma and the cat subsequently underwent exploratory laparotomy. The abdominal mass was located at one end of the spleen and was mottled pale brown to cream, roughly spherical, firm and raised above both surfaces of the spleen. Splenectomy was performed and histopathologic examination of the mass confirmed the diagnosis of splenic myelolipoma. Following surgery, the cat was quiet, alert and responsive but remained anorexic. Hyperthermia was detected and resolved by the day 3 of hospitalisation; dyspnea and abnormal lung sounds were however detected at the end of day 2 of hospitalisation and continued throughout day 3. The cat recovered from one resuscitation attempt but did not regain consciousness after a second attempt. Necropsy was not performed, and cardiac arrest was the suspected cause of death.

8: EVALUATION OF STA-PTTA AND STA-C.K.PREST REAGENTS, AND COMPARISON WITH STA-CEPHASCREEN FOR ACTIVATED PARTIAL THROMBOPLASTIN TIME MEASUREMENT IN DOGS, CATS, AND HORSES

Anne Geffré[1,2], Estelle Bernard Faye[1,2], Théo Chenal[1], Fanny Granat[1], Catherine Trumel[1,2]
[1]Ecole Nationale Vétérinaire de Toulouse, Toulouse, France, [2]Centre Régional d'Exploration Fonctionnelle et de Ressources Expérimentales (CREFRE), Toulouse, France

Background and Objective: The STA-Cephascreen reagent (Diagnostica Stago) was previously widely used in veterinary laboratories to measure activated partial thromboplastin time (aPTT). Its announced discontinuation prompted us to evaluate two alternative reagents, STA-PTTA and STA-C.K.PREST, on the STA-Compact Max3 analyzer for dogs, cats and horses.

Methods: For both candidate reagents, precision was tested by analyzing control plasmas (STA-COAG CONTROL N+P), twice in the morning and twice in the afternoon for 5 consecutive working days, and by analyzing the same citrated plasma 20 times within an hour for each species. We also compared the aPTT results obtained by the STA-Cephascreen and the two candidate reagents on 79 dogs, 60 cats and 64 horses requiring a haemostasis workup, using Passing-Bablok correlations, Bland-Altman plots and Spearman's estimators. Finally, we established new reference intervals for the retained reagent, using Passing-Bablok correlation equations.

Results: Using control plasmas, the coefficient of variation (CV) of repeatability was less than 1.2%, the intra-laboratory CV less than 2.4% and the bias less than 6% for both reagents. Using animal plasmas, CVs were below 3%, except for STA-PTTA, which had a CV of 10.5% in horses. Passing-Bablok equations and Bland-Altman plots revealed a moderate, proportional, negative bias for STA-C.K.PREST and a severe, proportional, positive bias for STA-PTTA. Spearman's rs was greater than 0.80 for STA-C.K.PREST and approximately 0.56 for STA-PTT A.

Conclusions: STA-C.K.PREST showed better analytical performance than STA-PTTA for the studied species. The new reference intervals for aPTT are [11.4-14.1]s in dogs, [11.0-15.1]s in cats and [36.8-46.2]s in horses.

9: PHAGOCYTIC FUNCTION AND FLOW CYTOMETRIC PHENOTYPE OF ASIAN ELEPHANT MONOCYTES

Jennifer Johns[1], Trinity Baumgartner[1], Carlos Sanchez[2], Brian Dolan[1]
[1]Oregon State University, Dept. of Biomedical Sciences, Carlson College of Veterinary Medicine, Corvallis, OR, USA, [2]Oregon Zoo, Portland, OR, USA

Introduction: Optimal veterinary care of managed elephant populations is increasingly vital due to the continued decline of wild populations. Appropriate health monitoring and accurate disease diagnosis include hematologic evaluation. Elephant hematology is distinctive in that elephants have

high percentages of monocytes in health. Elephant monocytes also have unusual morphology, a feature shared with manatees and rock hyraxes. The functional significance of these features is unknown. Manual white blood cell counting is used for elephant hematology as analyzers are generally inaccurate. The aims of this study were to evaluate basic cell isolation and functional testing protocols for use in elephant monocyte research, and to test several available antibodies via flow cytometry for use in elephant monocyte identification.
Methods: Peripheral blood samples from 5 Asian elephants (Elephas maximus) were used. Methods for monocyte isolation and evaluation of phagocytic function were evaluated. Putative lymphocyte and monocyte populations were identified via scatter on flow cytometry. Antibodies against CD11b, CD11c, CD14, and IBA1 were tested.
Results: Phagocytic function of elephant monocytes was visualized via fluorescence microscopy, and a kinetic spectrophotometric assay was established. Combined flow cytometric scatter and IBA1 positivity appear to identify Asian elephant monocytes, with other antibodies having lower apparent diagnostic utility.
Conclusion: This data provides a methodologic basis for further investigation into elephant monocyte function and immune response during clinically important infections, including tuberculosis and elephant endotheliotropic herpesvirus.

10: THE ADVIA 2120 MYELOPEROXIDASE CYTOGRAM DOES NOT DISTINGUISH ACUTE MYELOID FROM ACUTE LYMPHOBLASTIC LEUKEMIA IN DOGS

Mayzie Miller[1], Nora Springer[1], Dorothee Bienzle[2], Kristina Meichner[3], Tracy Stokol[4]
[1]University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, [2]University of Guelph Ontario Veterinary College, Guelph, ON, Canada, [3]University of Georgia College of Veterinary Medicine, Athens, GA, USA, [4]Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Acute myeloid leukemia (AML) cannot be reliably distinguished from acute lymphoblastic leukemia (ALL) by morphology. Different chemotherapeutic protocols may be recommended for AML versus ALL and immediate therapy is often needed due to rapid disease progression. Immunophenotyping may result in treatment delays; therefore, a rapid method for distinguishing AML from ALL at time of diagnosis is desirable. As myeloid cells are expected to have more myeloperoxidase activity than lymphoid cells, the ADVIA 2120 myeloperoxidase (MPX) cytogram may be able to differentiate AML from ALL.

Hypothesis: Large unstained cells (LUC) on the ADVIA 2120 MPX cytogram will create a more acute angle with the x-axis in AML versus ALL.

Methods: Dogs with acute leukemia were prospectively enrolled and phenotyped via flow cytometry and cytochemistry, including MPX, as part of a multi-institutional study. Fifty-eight dogs had available ADVIA MPX cytograms. A blinded observer estimated a line-of-best fit through LUC with FIJI software in triplicate and recorded the mean angle. Patients were then grouped as AML (N=43) or ALL (N=15) for comparison with a Mann-Whitney U test. Correlation between LUC angle and MPX cytochemical staining was assessed by Spearman Rank.

Results: There was no significant difference in the MPX LUC angle between AML and ALL (p=0.9). MPX cytochemical staining results did not correlate with LUC angle (R^{sp}=0.02 p=0.9). All cases with LUC angle <78°were diagnosed as AML (10/43, 23%).

Conclusions: The ADVIA MPX cytogram does not reliably distinguish between AML and ALL in dogs, although an angle <78° was suggestive of AML.

11: MICRORNA PROFILING IN CANINE CUTANEOUS AND SUBCUTANEOUS MAST CELL TUMORS

James A. Mori, Geoffrey A. Wood, Robert A. Foster, Anthony J. Mutsaers, R. Darren Wood
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Mast cell tumors (MCTs) are a common neoplasm in dogs, accounting for up to 21% of skin tumors. Although surgery is curative in most cases, some recur and/or metastasize, and thus have a poorer prognosis. More aggressive entities are treated with adjuvant radiation therapy and/or chemotherapy. Application of two- (grade I, II, or III) and three-tier (low- or high-grade) histopathologic grading schemes to excisional biopsy specimens remain the gold standard to determine prognosis for cutaneous MCTs. However, histopathology does not accurately predict the behavior of a subset of MCTs. Approximately 15% of dogs with histologically low-grade MCTs have local metastasis at diagnosis, thus making treatment decisions challenging. MicroRNAs (miRNAs) are short, non-coding, highly conserved oligonucleotides that post-transcriptionally silence genes and are dysregulated in neoplasia. Plasma miRNAs are promising biomarkers because they are highly stable and ubiquitous in tissue and blood. Little is known about the role of miRNAs in canine MCTs. This project aims to identify circulating plasma miRNAs correlating with clinical outcome and histopathologic grade. MiRNA profiles in plasma samples from dogs with different MCT grades were evaluated by quantitative PCR. Preliminary data show dramatic fold-regulation differences in several miRNAs between grade I, grade II/low, grade II/high, grade III, and subcutaneous MCTs. Notably, miRNAs previously implicated in canine MCTs have robust differential expression among these groups: miR-21-5p, miR-126, miR-214, and miR-26b. The results of this work will determine a miRNA “fingerprint” to assist in predicting clinical outcomes in MCTs of canine skin.

12: MULTIPLE MYELOMA WITH CRYOGLOBULINEMIA IN A DOBERMAN PINSCHER DOG

James A. Mori, Sébastien Sanz, Sanika Pandya, Gabriella Allegrini, Allison Collier, R. Darren Wood
Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Multiple myeloma (MM) is an uncommon plasma cell neoplasm associated with several paraneoplastic syndromes related to paraproteinemia. Monoclonal gammopathy in blood and/or urine, bone marrow plasmacytosis, or osteolytic lesions on imaging are diagnostic criteria for MM. Here, we describe a unique case of MM with cryoglobulinemia in a dog:

A 9-year-old female neutered Doberman Pinscher presented for acute blindness, persistent urinary tract infection, and polyuria/polydipsia. Physical examination revealed bilateral retinal detachment, conjunctival hyperemia, and central vestibular signs. Erythrocytosis (HCT: 0.66, reference interval [RI]: 0.39-0.66) was noted on the CBC. The biochemical profile showed hypoproteinemia (TP: 50 g/L, RI: 55-74), low-normal albumin (30 g/L, RI: 29-43 g/L), and hypoglobulinemia (20 g/L, RI: 21-42 g/L). Urinalysis showed isosthenuria with marked proteinuria (3+). Eight days later, HCT decreased to 0.36 and a sternal bone marrow aspirate revealed abundant basophilic proteinaceous material and marrow hypercellularity with marked plasmacytosis (~20% of hematopoietic cells). A distinct gelatinous layer formed in blood immediately after venipuncture, which was excluded on routine serum separation. The gelatinous layer was physically isolated, heated to 37°C (98.6°F), and diluted with fluidil (Sebia). TP of the the gelatinous material increased by 46 g/L and gel electrophoresis showed a narrow monoclonal peak in the beta-2 region, in contrast to the unremarkable electrophoretograms observed for untreated plasma and serum. A CT scan showed a splenic nodule and focal osteolysis of the cortex of the right 1st rib. The dog responded favorably to a single dose of cyclophosphamide and melphalan maintenance therapy.

13: CYTOLOGIC APPEARANCE OF A LYMPHANGIOSARCOMA WITH PULMONARY METASTASIS IN A CAT

Jacqui Nunnelley[1], Fred Williams[1], Kurt Williams[2], Andrew Miller[3], Hasan El Musa[1], Michelle DeCourcey[1]
[1]University of Missouri College of Veterinary Medicine, Columbia, MO, USA, [2]Oregon State University Carlson College of Veterinary Medicine, Corvallis, OR, USA, [3]Cornell University College of Veterinary Medicine, Ithaca, NY, USA

A 10-year-old female spayed, domestic shorthair cat presented for evaluation of a chronic cough. On physical examination, a firm mass was identified below the right mandible. Additional pertinent findings included tachypnea and increased bronchovesicular sounds. Aspirates from the mandibular mass contained atypical spindloid cells situated individually and in streaming bundles. These cells had eccentric, elongated nuclei with coarsely stippled chromatin and 1 to 2 prominent nucleoli. The cells had moderate to abundant amounts of blue cytoplasm. Moderate anisocytosis and marked anisokaryosis were observed. Occasional multinucleate cells were noted. Moderate numbers of small lymphocytes were observed associated with and surrounding the atypical mesenchymal cells. These findings were consistent with a sarcoma and the presence of many closely associated small lymphocytes raised the possibility of a lymphangiosarcoma. Due to the patient's decline, euthanasia was elected, and the patient was submitted for necropsy. Histologically, the mandibular mass was interpreted as a spindle cell neoplasm. The neoplastic cells were immunopositive for vimentin, CD31, PROX1, and lacked immunolabeling for factor VIII-related antigen. Histopathology of the lungs revealed metastatic soft tissue sarcoma with neoplastic cells following the distribution of lymphatics. The neoplastic cells in the lung were immunopositive for PROX1 and vimentin, and a majority of the cells lacked distinct immunolabeling for CD31 and factor VIII-related antigen. Immunopositive CD31 and PROX1 labeling in the mandibular mass confirmed a diagnosis of lymphangiosarcoma with pulmonary metastasis. Lymphangiosarcoma is an uncommon neoplasm in cats. The cytologic description of neoplastic cells from IHC-confirmed lymphangiosarcoma is rarely described in veterinary literature.

14: LYMPHOCYTE CYTOMORPHOMETRIC CHANGE IN BLOOD AND LYMPH NODE WITH CANINE AND FELINE LYMPHOPROLIFERATIVE DISEASE

Peter O'Brien
University College Dublin, Dublin, Ireland

Background: We previously characterized cytomorphometric changes in nodal lymphocytes with lymphoma (LSA) in cats and dogs. Increased, maximal cell-length (CL,um) was most simply and reliably discriminating. Here, we tested whether CL increased: a) with non-malignant, nodal changes of inflammation (IFL) or plasma cell hyperplasia (PCH) in dogs; and, b) in peripheral blood lymphocytes (PBL) of dogs and cats with LSA. **Methods**: Mean CL was determined for: a) 25 intact lymphocytes across 3 randomly-selected fields-of-view in node smears for canine cases with medium-to-large cell LSA (LCL); hand-mirror LSA (HML); normal (Nor); IFL; PCH; IF+PCH. b) or for the largest 10 intact lymphocytes in blood smears of dogs and cats with LCL or lymphocytic leukemia (LL). Data as mean±SD,range,n. Comparisons by ANOVA and t-test. All $p<0.05$. **Results**: a) %CL>11um were, respectively: 66.8±15,30-92,50; 1.5±1.2,0.0-0.6,17; 3.8±3.5,0-12,25; 5.7±5.5,1-21,20; 9.3±7.0,0-22,16; 5.6±3.6,2-14,19. Compared to Nor, %CL>11um increased 18-fold in LCL and 2.5-fold in PCH. b) PBL CL of 13 cats and 32 dogs (Nor) was 11.5±0.5,10.5-12.6,45, but mildly higher in dogs with benign, reactive lymphocytosis or reactive PBLs (12.0±0.4,12.4,11.2-12.7,14). LSA in 29 dogs and 10 cats (13.9±1.1,12.4-16.9,39), was higher than both Nor and benign reactive cases: and 14.9±1.2,13.5-16.9,10 in canine LL. Using 12.6um CL as cut-off for 111 canine and feline cases >90% with, and >95% without, malignan lymphocytic change were accurately identified. **Conclusion**: Lymphocyte CL (%>11um) in lymph nodes separated normal, inflammatory and reactive changes

from all lymphomas (LCL). Largest PBL CL identified 90% lymphomas and leukemias from cases with normal and reactive lymphocytes.

15: IMPACT OF MAGNIFICATION, IMAGE TYPE AND COUNT ON CONVOLUTIONAL NEURAL NETWORK PERFORMANCE IN DIFFERENTIATING CANINE LARGE CELL LYMPHOMA FROM NONLYMPHOMA VIA LYMPH NODE CYTOLOGY

Christina Pacholec, Hehuang Xie, Julianne Curnin, Amy Lin, Kurt Zimmerman
Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Background: Lymph node (LN) aspirates used to evaluate canine lymphadenopathy can often differentiate canine large-cell lymphoma (LCL) from benign processes.

Objective: This study used images of LN cytology to test the effects of image magnification, type, and number on the performance of a convolutional neural network (CNN) to differentiate canine large-cell lymphoma (LCL) from benign processes.

Methods: LN cytology was imaged from 150 patients diagnosed with LCL and 150 patients with nonlymphoma (NL). Three hundred images of LCL and NL were used to train a CNN and interrogate the effects of magnification, image type, and image number on the model's performance.

Results: The model performance was highest for color images using a 100x objective, with an accuracy of 95.8%, ROC area of 0.997, and F-measure of 0.958, and for greyscale images taken at 100x objective, with an accuracy of 96.67%, ROC area of 0.994, and F-measure of .967.

The number of images used affected the model's performance. Performance increased most significantly as the total number of images increased from 200 to 300. Accuracy, ROC area, and F-measure for 200 images measured 70%, 0.768, and 0.694, while for 300 images, they were 95%, 0.939, and 0.95, respectively.

Conclusion: For CNN image classification tasks with LN cytology, using color versus greyscale images does not significantly affect the model's performance. The magnification that yields the highest-performing model is 1,000x. Lastly, 150 images per class (300 total) for a 2-class problem is the minimum number recommended for pilot studies using CNNs for performance estimates.

16: METHEMOGLOBINEMIA CASE SERIES

Jeanine Sandy[1], Stephanie Johnnides[2], Toby Chu[1]
[1]City University of Hong Kong, Hong Kong, Hong Kong, [2]Harvest Veterinary OncologyCentre LTD, Hong Kong, Hong Kong

This case series describes six patients (2 cats and 4 dogs) with methemoglobinemia and associated clinical signs manifesting as peracute tachypnea, respiratory distress, tachycardia, and cyanosis. Patients were presented to a veterinary emergency center for assessment, including point-of-care testing with co-oximetry, which rapidly confirmed methemoglobinemia. Methemoglobinemia varied from mild to severe (8.4 – 70.4%) and typically fell to <3% within 24 hours of treatment. All animals responded well to standard therapies with N-acetylcysteine, except for one cat, who died of complications associated with acute acetaminophen toxicity. Methemoglobinemia was caused by accidental ingestion of hydroxyurea in one dog, suspected to be induced following ingestion of a novel pet treat, but the trigger was unknown for the remaining three patients. This report highlights the need for clinicians to be aware of methemoglobinemia as a cause of peracute cyanosis with brown discoloration of blood or urine, to aid prompt medical attention.

17: CYTOLOGIC AND HISTOLOGICAL FEATURES OF A PSAMMOMATOUS MENINGIOMA IN A DOG

Michael Talavera, Kayla Alexander, Andy Shores, Marjorie Williams, Matthew Williams
Mississippi State University College of Veterinary Medicine, Starkville, MS, USA

An 8-year-old neutered male mixed breed dog presented to Mississippi State University College of Veterinary Medicine for evaluation of seizures. The patient's first seizure occurred four years prior to presentation, with increasing duration and frequency. On neurological examination, the patient was mentally dull, laterally recumbent, with no menace or gag reflex, resulting in a coma score of 10/15 (Small Animal Coma Scale). MRI revealed an extra-axial, smoothly marginated, ovoid to irregularly shaped mass measuring 1.5 x 1.4 x 1.8 cm located along the rostrodorsal aspect of the left frontal bone which was T2/T1 FS/FLAIR isointense to the gray matter and strongly contrast-enhancing. The patient underwent a frontal craniotomy and samples of the mass were submitted for cytological and histological examination. The impression smears showed a moderate number of neoplastic meningeal cells. There were several round to oval, variable-sized, crystalline-like structures with basophilic to lightly amphophilic lamellated cores embedded within neoplastic cellular aggregates, tentatively identified as psammoma bodies, with the cytological interpretation being suspected psammomatous meningioma. Histologically, the mass was composed of numerous spindle cells arranged in streams and concentrically laminated whorls of hyaline material with frequently mineralized cores, consistent with a psammomatous meningioma. Within the veterinary literature, there are limited cytological case reports of psammomatous meningiomas with readily identifiable psammoma bodies. Given the increased utilization of intra-operative impression smears, it is vital to document distinguishing cytological features of central nervous system neoplasms. This case highlights that cytological evaluation can definitively and accurately diagnose psammomatous meningioma.

18: CYTOLOGICAL APPEARANCE OF SPLENDORE HOPPLI REACTION

Harold Tvedten
Swedish University of Agricultural Sciences, Uppsala, Sweden

Splendore-Hoeppli phenomenon (SHP) is a splendid and beautiful inflammatory reaction best known for its histological appearance, as for example in lumpy jaw disease (*Actinomyces bovis*) in cattle. A case of Actinomyces skin granuloma in a Guinea pig offered exceptionally good examples of how to identify the SHP on cytological examination. The filamentous forms of Actinomyces were coated with a variably thick layer of material. This Spendore-Hoeppli (SH) material stained not only red (eosinophilic) as usual but even blue with Giemsa stain. The SH coated Actinomyces filaments were often phagocytized by macrophages and even few neutrophils (heterophils). Histological sections of the excised granuloma had the classical bacterial colonies (sulfur granules) with a ring of eosinophilic material around them inside a pool of neutrophils. SH material on filaments of Actinomyces extending from the large bacterial colony were brightly red and club-shaped. The case also demonstrated well the second, less known morphological (diptheroid) form of Actinomyces as small pleomorphilc cocco-bacillary bacteria.

19: BILE SAMPLE EVALUATION IN DOGS AND CATS: RETROSPECTIVE STUDY OF 82 CASES

Pâmela Valente[1,2,3], José Henrique Correia[1,2], Maria Joana Dias[1,2,3]
[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, [2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal, [3]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

Introduction: Bile assessment is important in the investigation of hepatobiliary diseases in small animals, however literature about it is scarce.

Objective: To evaluate and describe the cytology and bacterial culture of bile samples from small animals.

Methods: A retrospective study was conducted reviewing bile samples collected by cholecystocentesis from dogs and cats with hepatobiliary disease, between 2020 and 2024, in a Veterinary Hospital. Data regarding signalment, cytological examination and bacterial culture was recorded and analyzed using descriptive statistics.

Results: Eighty-two bile samples were collected: 53 (64.6%-53/82) from cats (27 females; 26 males) and 29 (35.4%-29/82) from dogs (20 males; 9 females). The media age was 9.8(±4.3) years for cats and 10.1(±3.6) years for dogs. All bile samples (100%-82/82) underwent cytological examination and 75 (91.5%-75/82) underwent bacterial culture. Cytological evaluation was normal in 64.1% (34/53) of cats and 82.8% (24/29) of dogs. Bacteria on cytology was detected in 17.0% (9/53) of cats and 17.2% (5/29) of the dogs. Inflammation without bacteria, crystals and parasite eggs were observed only in cats, namely in 15.1% (8-53), 7.5% (4/53) and 1.9% (1/53) of cases, respectively. All bile with cytological detected bacteria were positive in culture. Bile samples without bacteria identified on cytology cultured positive in 6.9% (2/29) of cats and 9% (1/22) of dogs. Escherichia coli was the most commonly isolated agent in both species.

Conclusions: Most bile cytological examinations were normal. All bile with cytological detected bacteria had positive cultures. However, some false negatives were identified by bacterial culture, which highlights its importance.

20: PRESUMPTIVE AML-M6ER WITH MARKED RUBRICYTOSIS AND DISCORDANT FELV STATUS IN A DOMESTIC SHORTHAIR CAT

Rachel Whitman, Rose Nolen-Walston, Kathryn Shaffert, Stephanie Kline, Caitlin Jozwiak, Carrie Wood, Francisco Conrado
Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA

An 8-year-old neutered male domestic shorthair cat was presented for evaluation of a 2-week history of decreased appetite and hiding. He had been adopted at 4 months oldd and kept indoors since. Prior medical history included well-controlled hypertrophic cardiomyopathy with biventricular congestive heart failure and chronic hematuria of uncertain etiology. A complete blood count revealed a marked non-regenerative anemia (PCV 13%; RI 30-50). On blood film review, there were numerous (64.4 K/µL) morphologically unremarkable erythroid precursors, primarily metarubricytes with fewer rubricytes and prorubricytes, mild anisocytosis, and absent polychromasia. Point-of-care tests for feline immunodeficiency virus antibody and feline leukemia virus (FeLV) p27 antigen were negative. Abdominal ultrasound did not identify an obvious cause for the anemia or hematuria. Marked hyperplasia, a left shift, and incomplete maturation of the erythroid lineage were observed on bone marrow aspirate cytology and core histopathology with minimal dysplastic change. The patient's peripheral blood and bone marrow findings were considered most consistent with acute myeloid leukemia, erythroid subtype (AML-M6Er). An FeLV antigen immunofluorescence assay (IFA), performed on bone marrow aspirate smears, was positive. Chemotherapy was administered, though the cat was euthanized the next day due to acute clinical decline. A diagnosis of AML-M6Er is appropriate when early erythroid precursors comprise >50% of marrow cells and blast cells are >30% of all nucleated cells. It is commonly associated with FeLV infection in cats. This case illustrates an interesting manifestation of AML-M6Er with marked rubricytosis and discordant FeLV test results.

21: CORRELATING DIGITAL CYTOPATHOLOGY AND NEOPLASTIC CAVITARY EFFUSION IN A 12-YEAR-OLD CAT

Paulina Wozniak, Stacey Newton
Nationwide Laboratories, Poulton-le-Fylde, United Kingdom

An entire female 12-year-old domestic shorthair cat was presented for clinical evaluation and diagnosed with a cranial abdominal mass, suspected of pancreatic origin, and evidence of peritoneal fluid. Fine-needle aspirates of the mass and peritoneal fluid were submitted to a UK veterinary diagnostic laboratory. The samples were prepared using cytospin technique and assessed using digital cytopathology.

The predominant population from the mass consisted of moderately to markedly pleomorphic epithelial cells, with round nuclei, finely stippled chromatin, prominent nucleoli and moderate - abundant basophilic cytoplasm. The cells were frequently distributed in clusters and occasionally presented pseudo-acinar arrangements, highly suggestive of adenocarcinoma. The slides from the peritoneal effusion revealed similar population of cells, admixed with a mixed inflammatory component.

Pancreatic adenocarcinoma is uncommon in cats; however, it is the most common tumor of the exocrine pancreas. There are no breed or sex predispositions. The clinical signs may include vomiting, abdominal pain, weight loss. The most common sites for metastasis are liver, peritoneum and local lymph nodes. The median survival time of cats with abdominal effusion is approximately 30 days compared with 165 days for cats with no abdominal effusion. Furthermore, sampling of pancreatic tumors in cats can have the added risk of transabdominal seeding of tumor cells. This report reinforces the fact that pancreatic carcinoma should be rated highly in the differential diagnosis in mature adult and senior cats with cranial abdominal masses, ascites and/or jaundice and the benefit of early diagnostics with cytology can steer further therapeutics in affected individuals.

Experimental Disease Posters

23: IMPORTANCE OF INFECTION ORDER OF PORCINE CIRCOVIRUS TYPE 2D AND MYCOPLASMA HYOPNEUMONIAE

Chanhee Chae, Jeongmin Suh, Sehyeong Ham
Seoul National University, Seoul, Republic of Korea

This study compared the different sequential order of infection of porcine circovirus type 2d (PCV2d) and *Mycoplasma hyopneumoniae*. Thirty-six pigs were allocated randomly across six different groups. Pigs underwent various inoculation sequences: *M. hyopneumoniae* administered 14 days before PCV2d, simultaneous PCV2d-*M. hyopneumoniae*, PCV2d given 14 days before *M. hyopneumoniae*, PCV2d only, *M. hyopneumoniae* only, or a mock inoculum. Overall, the pigs inoculated with *M. hyopneumoniae* 14 days prior to PCV2d (Mhyo-PCV2 group) and those inoculated simultaneously with PCV2d and *M. hyopneumoniae* (PCV2+Mhyo group) displayed notably higher clinical disease severity and experienced a significant decrease of their average daily weight gain than pigs inoculated with PCV2d 14 days prior to *M. hyopneumoniae* (PCV2-Mhyo group). *M. hyopneumoniae* infection potentiated PCV2 blood and lymph node viral loads, as well as PCV2-associated lesions, while the infection of PCV2d did not impact the intensity of *M. hyopneumoniae* infection. Tumor necrosis factor-alpha sera levels were significantly increased in the Mhyo-PCV2 and PCV2+Mhyo groups as compared to the PCV2-Mhyo, PCV2, and Mhyo groups. The most important information was that the potentiation effect of *M. hyopneumoniae* on PCV2d was found only in pigs inoculated with either *M. hyopneumoniae* followed by PCV2d (Mhyo-PCV2 group) or a simultaneous inoculation of PCV2d and *M. hyopneumoniae* (PCV2+Mhyo group). The sequential infection order of PCV2d and *M. hyopneumoniae* resulted in divergent clinical outcomes.

24: TNF-A AND TH2 CYTOKINES PROMPT CANINE ATOPIC DERMATITIS–LIKE MORPHOLOGIC AND MOLECULAR CHARACTERISTICS IN THE CANINE EPIDERMAL ORGANOID CULTURE SYSTEM

Bo Chen, Smitha Georgy, Ronald Slocombe
The University of Melbourne, Melbourne, Australia

Canine Atopic Dermatitis (CAD) is a chronic inflammatory and pruritic skin disease that affects up to 27% of the canine population. This frequently encountered disease leads to discomfort and reduced quality of life in affected animals. The pathogenesis of CAD remains incompletely understood, involving a complex interplay of inflammatory mediators, diverse genetic backgrounds influencing skin barrier formation, the cutaneous microbiome, and environmental factors. This complexity leads to variable responses to therapy. Thus, similar to Atopic Dermatitis (AD) in humans, dogs with CAD require individually-tailored treatment.

Our methodology utilizes a well-controlled model system known as the canine epidermal organoid system, which is derived from normal canine keratinocytes and exhibits morphological characteristics and key marker proteins consistent with normal canine skin. We investigated the direct impact of specific immune mediators, namely IL-4, IL-13 (Th2 cytokines), and TNF-α (a pro-inflammatory cytokine), both individually and in combination, on skin barrier components using this model system.

The results demonstrated that TNF-α and Th2 cytokines induce CAD-like morphological and molecular characteristics in the canine epidermal organoid system, including epidermal spongiosis, decreased epidermal differentiation, and reduced expressions of filaggrin, involucrin, and loricrin, along with the signs of proliferation and apoptosis. These findings indicate that the canine epidermal organoid system holds promise as a valuable tool for understanding the pathogenesis of CAD and can become a potential platform for assessing individual-specific treatment options or screening drug candidates for each atopic case.

25: TRANSLATIONALLY RELEVANT CARDIOVASCULAR AND NEURAL BIOMARKERS: FROM SWINE TO HUMANS

Hayden Dillow
CBSET, Lexington, MA, USA

There is an unmet medical need to treat cardiovascular disease, due to the prevalence of individuals who do not respond to treatments despite recent medical advancements. Specifically, resistant hypertension and myocardial infarct are a great concern. Treatment of resistant hypertension includes three or more antihypertensives, surgical intervention, and lifestyle changes. For surgical interventions, renal denervation can decrease blood pressure by affecting the sympathetic nervous system. For myocardial infarct, a new treatment was developed to protect cardiac tissue from ischemic injury

This presentation will discuss the validity of using swine models for pre-clinical testing and research for cardiovascular disease. It will also highlight the use of systemic, imaging, and postmortem biomarkers that can validate results from the model. Lastly, it will discuss the translational relevance of using the swine model as a surrogate for what occurs in the clinic setting through imaging and surgical procedures.

Two types of studies were performed. One involved renal denervation in swine models, and the other involved cardioprotective dosing of a compound for myocardial infarct. For the renal denervation study, renal nerves were ablated with nerve mapping and norepinephrine analysis performed. For the infarct model, intravenous dosing of an insect derived product was performed. In-life imaging and postmortem examinations were performed to determine the effect of the treatment.

The results showed a positive correlation between norepinephrine and nerve ablation while the intravenous dosing showed no statistical difference in the infarct model. These studies show swine to be a useful translational model for cardiovascular disease states.

26: STAPHYLOCOCCUS AUREUS TOXIC SHOCK SYNDROME TOXIN PROMOTES ATOPIC DERMATITIS INDEPENDENT OF HUMAN MHC CLASS II BINDING

Laine Feller, Sabrina Kline, Jing Zhang, Meng-Jen Wu, Yu Wang, Nathan Archer
Johns Hopkins University School of Medicine, Baltimore, MD, USA

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease of childhood, and is associated with *Staphylococcus aureus* overcolonization. Staphylococcal superantigens (SAgs), such as Toxic Shock Syndrome Toxin (TSST), are implicated in AD progression, but their mechanism of action in the skin is unknown. In other contexts, SAgs induce oligoclonal T cell proliferation by nonspecifically cross-linking MHC class II (MHCII) and T cell receptors independent of their antigen specificity. To elucidate SAgs' pathogenicity in skin and dependency on MHCII binding, we performed a mouse model of AD wherein $1x10^8$ CFU of parent or TSST-knockout (ko) *S. aureus* was applied to the shaved and depilated dorsal skin for 7 days. As SAgs have selective affinity for human MHCII over mouse, all experiments were performed in wild type C57BL/6J and transgenic HLA-DR4 mice, which exclusively express human MHCII. Two independent replicates were performed per experiment, with 4-5 mice in each replicate treatment group. We found that HLA-DR4 mice colonized by TSST-ko *S. aureus* had a 15µm reduction in epidermal thickness ($P<0.01$), five-fold reduction in serum IgE ($P<0.0001$), and reduced neutrophil, macrophage and T cell infiltration into skin (P values<0.05 to<0.01) compared to the parent control. These results repeated in C57BL/6J mice, as mice colonized by TSST-ko *S. aureus* exhibited similar reductions in epidermal thickness, IgE, and immune cell infiltration relative to the parent control as when performed in HLA-DR4 mice. Collectively, our data suggests that TSST promotes AD pathogenesis via a non-classical SAg-binding mechanism that is independent of human MHCII binding.

27: HAMSTERS DISPLAY VARIOUS DISEASE STAGES OF COVID-19 REPRESENTING POTENTIAL KEY ELEMENTS OF LONG-COVID

Laura Heydemann[1], Malgorzata Ciurkiewicz[1], Theresa Stoerk[1], Kirsten Hülskötter[1], Katharina Gregor[1], Tom Schreiner[1], Wencke Reineking[1,2], Asisa Volz[1,3], Maren von Koeckritz-Blickwede[1,3], Robert Geffers[4], Klaus Schughart[5,6], Dirk Schaudien[7], Wolfgang Baumgärtner[1], Federico Armando[1,8]
[1]University of Veterinary Medicine, Hanover, Germany, [2]Stanford University, Stanford, CA, USA, [3]Research Center for Emerging Infections and Zoonoses (RIZ), Hanover, Germany, [4]Helmholtz Centre for Infection Research (HZI), Brunswick, Germany, [5]University of Muenster, Muenster, Germany, [6]University of Tennessee, Memphis, TN, USA, [7]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hanover, Germany, [8]University of Parma, Parma, Italy

Background:

The Syrian hamster (*Mesocricetus auratus*) is an established animal model for studying the acute and early subacute phase of *COVID-19* in humans. However, little is known about the advanced stages of the disease in this animal model and its potential role for investigating the pathogenesis of *long COVID*.

Objective:

We aimed to characterize the subacute and chronic features of the respiratory SARS-CoV-2 infection in hamsters using light microscopy as well as functional and transcriptomic analyses.

Methods:

112 male, 11- to 12-months old hamsters were infected intranasally with the SARS-CoV-2-Delta variant or received PBS. Functional data were obtained using whole body plethysmography before

and after exercise on a rodent treadmill. Animals were sacrificed at 1, 3, 6, 14, 28, 56 and 112 days post infection (dpi). Lungs were examined by histochemistry, immunohistochemistry, and analyzed for transcriptomic changes.

Results:

Infected animals showed three distinct stages of the disease: The acute phase (infection - 6 dpi) was characterized by virus infection, inflammation and marked, but transient decrease of pulmonary function. This was followed by a subacute period (14 dpi) with complete virus clearance, alveolar regeneration as well as interstitial and subpleural fibrosis. In the chronic phase (28 - 112 dpi), lungs showed persistent fibrosis and multifocal alveolar epithelial proliferations, accompanied by transient, exercise-induced pulmonary dysfunction until 42 – 49 dpi.

Conclusions:

Exercise-induced pulmonary impairment, chronic interstitial fibrosis, and alveolar bronchiolization in SARS-CoV-2-infected Syrian hamsters may represent key elements for understanding the pathogenesis of *long COVID* in human patients.

28: SINGLE-CELL RNA-SEQUENCING OF THE MUTANT IL-7R-RASGRP1-DRIVEN MODEL OF T CELL ACUTE LYMPHOBLASTIC LEUKEMIA REVEALED POTENTIAL MECHANISMS OF ONCOGENE COOPERATION

Steven Hsu[1,2,3], Gisele Rodrigues[2], Julie Hixon[2], Wenqing Li[2], Scott Durum[2]
[1]Comparative Biomedical Scientist Training Program, National Institutes of Health, Bethesda, MD, USA, [2]National Institutes of Health, National Cancer Institute, Frederick, MD, USA, [3]Michigan State University, College of Veterinary Medicine, East Lansing, MI, USA

Approximately 10 percent of T cell acute lymphoblastic leukemia (TALL) cases harbor mutations in the IL-7 receptor (mutIL7R) that render the receptor constitutively active; however, mutIL-7R alone is insufficient to transform thymocytes. A second oncogene, such the overexpression of RasGRP1, c-myc, TLX3, or mutant Nras, cooperates with mutIL-7R to drive leukemogenesis. It is unclear how the addition of RasGRP1 overexpression affects gene expression to drive leukemogenesis. 5’ library construction and single-cell RNA sequencing were performed on splenocytes derived from Rag mice injected with mutIL-7R- or mutIL-7R+RasGRP1-transduced C57BL/6-derived thymocytes. Suppression of *Socs1* ($p<0.001$) and *Jund* ($p<0.001$) were observed in the mutIL-7R+RasGRP1 group compared to mutIL-7R alone group. Mechanistically, Socs1 is a key negative regulator of the JAK-STAT pathway, and JunD is known to suppress transformation by activated Ras. Thus, the suppression of *Socs1* and *Jund* by the mutIL-7R-RasGRP1-driven leukemia may represent the mechanism by which mutIL-7R and RasGRP1 cooperate to drive leukemogenesis.

29: AGE-DEPENDENT CHANGES IN MOUSE CORNEAL NERVE FIBERS USING A NOVEL THREE-DIMENSIONAL QUANTITATIVE ANALYSIS

Minori Inanaga Kojima, Tetsuro Matsuura, Ayumi Matsuda, Kiyokazu Ozaki
Setsunan university, Osaka, Japan

Introduction Corneal nerve fibers (CNFs) are crucial for corneal sensation and maintenance, and quantification of CNFs can indicate peripheral nerve damage from disease and aging. However, few studies have analyzed the intracorneal nerve endings (ICNTs) in three dimensions (3D) .

Objective To determine the 3D structure of mouse CNFs and quantify age-related changes.

Methods Male C57BL/6 mice aged 8 to 104 weeks were used. Corneal nociceptive responses were measured, and CNFs were visualized using transparency techniques and immunofluorescence. Confocal microscopy and Imaris software were employed for 3D reconstruction and quantitative analysis of CNFs.

Results An age-related decline in corneal nociceptive response was observed. The 3D image of CNFs revealed that the intracorneal basal nerve plexus (ICBN) branched from the stromal nerve bundle in the corneal periphery. ICBN formed a spiral towards the corneal center and subsequently branched out to form ICNTs. ICNTs extended perpendicularly and terminated within the epithelial layers, predominantly composed of simple fibers, with some intricate ones. Quantitative analysis demonstrated significant decreases in total lengths and densities of ICBN and ICNTs from 8 to 26 weeks. After 26 weeks, ICNTs continued declining until 104 weeks, while ICBN remained stable. ICNTs showed a significant decrease in total number and an increase in individual fiber length up to 26 weeks. Individual fiber length decreased from 26 weeks to 104 weeks.

Conclusion This study provided precise 3D insights into CNFs, revealing age-related reductions in mouse corneal nerves. Notably, ICNT numbers decreased until mature adult, with lengths declining into old age.

30: IMMUNOTHERAPY ON DEMAND: TAILORING A PD-1-DEPENDENT 4-1BB AGONIST FOR COMBINATION IMMUNOTHERAPY

Alana Kramer, Damien Ruiz, Jonathan Marable, Maninder Sandey
Auburn University College of Veterinary Medicine, Auburn, AL, USA

Background: Immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4) and immunostimulatory agonists (4-1BB, OX40) have emerged as promising cancer immunotherapies. These therapies are developed to enhance cancer patient's immune response, especially to refractory treatments. Despite their proven therapeutic efficacy, 4-1BB agonists elicit a dose-limiting hepatotoxicity by recruiting CD8+ T-cells to the liver in preclinical and clinical trials. Restriction of 4-1BB activation to the tumor microenvironment (TME) is hypothesized to limit hepatotoxicity while simultaneously triggering a robust antitumor response.

Objectives: To develop and evaluate a set of bispecific immunostimulatory fusion protein constructs that consolidate PD-1 blockade and 4-1BB activation into a single therapeutic and activate 4-1BB signaling in the TME, only in the presence of PD-1 expressing T-cells.

Methods: Three murine specific bispecific fusion proteins were engineered, expressed and purified using Expi293-F cells. The binding ability of the bispecific fusion proteins to murine PD-1 and 4-1BB was evaluated using flow cytometry. Moreover, the ability of the bispecific fusion proteins to disrupt PD-1/PD-L1 interaction and activate 4-1BB signaling in a PD-1 dependent manner was evaluated using functional assays.

Results and **Conclusions:** All three bi-specific proteins were successfully purified and showed high binding affinity and specificity to murine PD-1 and 4-1BB receptors. They also successfully inhibited PD-1/PD-L1 interaction in a cell-based assay and activated 4-1BB signaling in a PD-1 dependent manner. Future *in vivo* studies aim to test therapeutic efficacy and toxicity profiles in a mouse melanoma tumor xenograft model.

31: DIFFERENTIAL INFLAMMATORY PROFILES IN ADULT AND GERIATRIC COTTON RATS INFECTED WITH RESPIRATORY SYNCYTIAL VIRUS

Jonathan Miller, Cameron Leedale, Stefan Niewiesk
The Ohio State University, Columbus, OH, USA

Human respiratory syncytial virus (RSV) is a leading cause of severe respiratory disease in elderly populations. RSV clearance is delayed in individuals over 65 years of age, a finding that is recapitulated in the geriatric cotton rat model. Altered inflammatory responses in elderly populations have been implicated with impaired clearance of several pathogens but remain poorly described for RSV. Lung histology and bronchoalveolar lavage cytology do not show significant differences between adult and geriatric cotton rats infected with RSV, but these methods do not assess the molecular inflammatory environment. We performed RNA sequencing (RNA-seq) and pathway analysis on lung of RSV-infected adult and geriatric cotton rats to characterize differential host immune responses between these age groups. No differences in inflammatory gene expression were observed between uninfected adult and geriatric cotton rats. At day 1 post-infection, adult cotton rats expressed higher levels of several cytokines and transcription factors associated with acute inflammation and initiation of a Th1 response, including IFN-beta, IL12, and Tbet, compared to geriatric cotton rats. By day 4 post-infection, adult cotton rats expressed higher levels of effector molecules associated with Th1 responses, including IFN- gamma. Pathway analysis showed significantly increased activation of several pro-inflammatory pathways in adults, including the Th1 pathway, macrophage classical (M1) activation, and pathogen-induced cytokine signaling. Geriatric cotton rats expressed higher levels of several genes associated with macrophage alternative (M2) activation. Collectively, these results indicate that geriatric cotton rats exhibit impaired generation of a Th1 response to RSV.

32: HARNESSING EPITHELIAL-MESENCHYMAL PLASTICITY TO SENSITIZE QUASI-MESENCHYMAL BREAST TUMORS TO IMMUNE CHECKPOINT BLOCKADE THERAPY

Sophie Nelissen[1], Isabel O'Connell[1], Shiney Chandraganti[1], Anushka Dongre[1,2]
[1]Dept. of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA, [2]Adjunct Dept. of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Epithelial-mesenchymal transition (EMT) drives immunosuppression and resistance to immune checkpoint-blockade (ICB) therapy in breast cancers. TGF-β1, a major regulator of EMT, is widely immunosuppressive, making it an attractive target to reverse EMT and restore tumor immunogenicity. We compared two murine mammary cancer cell lines: a quasi-mesenchymal cell line (pB3, MMTV-PyMT background), and pB3 cells knocked out for TGF-β1 (sgTGF-β1). The sgTGF-β1 cell line was generated using CRISPR/Cas9 (plasmid: TGFB1; SC-423364). The absence of TGF-β1 was confirmed by ELISA. *In vitro*, immunofluorescence (IF) demonstrated strong cytoplasmic expression of E-cadherin and vimentin in sgTGF-β1 cells, whereas pB3 cells mostly expressed vimentin. Parental and sgTGF-β1 cells were orthotopically injected in the inguinal mammary fat pad of immunocompetent, syngeneic C57BL6/J mice. One untreated group and one group treated with anti-CTLA4 ICB were defined per condition (intraperitoneal injection: 200µg/mouse, 3x/week; n=3 mice/group). Tumor growth was monitored using vernier calipers 2x/week and tumors weighed at endpoint. Histologically, neoplastic cells were spindloid and formed interlacing bundles in all groups. Tissue IF subjectively demonstrated increased numbers of CD4+ and CD8+ T-cells within sgTGF-β1 tumors, and large numbers of M2-macrophages across all groups (F4/80+Arg1+). A one-way ANOVA test ran on flow cytometry data demonstrated significantly increased numbers of CD3+ ($p=0.0381$) and CD4+ ($p=0.0062$) T-cells in untreated sgTGF-β1 mice compared to untreated pB3. However, tumor sizes and weights remained similar across all groups. We thus conclude that abrogating TGF-β1 partially reverses EMT and restores T-cell infiltration but does not affect the presence of M2-like macrophages, which may compromise ICB responsiveness.

33: THE INFLUENCE OF AIR BARN PARTICLE EXPOSURE ON THE ACTIVATION OF BOVINE MACROPHAGES

Zahra Nikousefat, Jeff Caswell
Ontario Veterinary College, Guelph, ON, Canada

Air quality in barns is a critical factor affecting the respiratory health of livestock. This study examines how barn air particles affect the activation of bovine macrophages. Bovine monocyte-derived macrophages were harvested differentiated with GM-CSF, then exposed to collected air particles from Ontario calf barns. Sixteen calves were placed in a room with good air quality and sampled. Then, the ventilation was adjusted to achieve poor air quality, and different calves were sampled at subsequent times. Each calf was sampled before and 24 hours after receiving an aerosol of killed bacterial lysate as an inflammatory stimulus. Post-exposure total cell counts and neutrophils percentage were measured from bronchoalveolar lavage fluid. Result showed exposure to air barn particles led to a dose-dependent (0.317 to 1µgr /ml) activation of MDMs after 24 h of exposure. Particle sizes of 1.1-3.3 µm initiated the greatest macrophage activation, including increased size, granularity and cell death. This activation also included secretion of the pro-inflammatory cytokines IL-1β, and IL-8 ($P<0.0001$). Lung inflammation induced by poor air quality revealed increased neutrophils (49.88± 4.59/ ml3) and total counts (1092± 74.2/ml3) in BALF samples ($P=0.0136$). The mechanisms of inflammation may include the effects of air particles, ammonia or humidity on the survival of pathogens. Chemokines are secreted in response to barn air particulates and facilitate the recall of immune cells to the site of inflammation. These findings highlight the importance of macrophage activation on bovine respiratory health. Ongoing work will determine the expression and secretion of pro-inflammatory markers on lung macrophages.

34: EOSINOPHILIC INFLAMMATION THAT BEGINS IN THE JUVENILE STAGE CAUSES HYDRONEPHROSIS AND UROTHELIAL CANCER IN MUTANT MICE

Kiyokazu Ozaki, Yui Terayama, Minori Inanaga Kojima, Tetsuro Matsuura
Setsunan University, Hirakata, Japan

Background: Obstructive hydronephrosis is caused by various factors such as chronic inflammation and tumors. Eosinophils and chitinase-like proteins (CLPs) are involved in the pathogenesis of hydronephrosis in mice; however, the specific mechanisms remain unknown.

Objective: This study aimed to investigate a novel mouse model (B6-H) of obstructive hydronephrosis from onset to progression to clarify the effects of eosinophils and CLP on the development of hydronephrosis and tumorigenesis.

Methods: The ureteropelvic junction of B6-H mice were histologically analyzed in comparison with control B6 mice at 1-8 weeks of age to clarify the relationship between uroepithelial proliferation and inflammation. At 60 weeks of age, neoplastic change at the same ureteropelvic junction was also examined.

Results: The primary change was slight eosinophil infiltration of the ureteropelvic junction, even at 1 week of age, followed by a significant increase in CLP expression in the urothelium at 5 weeks of age, which led to proliferation of the urothelium. At 8 weeks of age, polyps with eosinophilic inflammation and urothelial hyperplasia expressing high levels of CLP formed at the ureteropelvic junction, leading to hydronephrosis. At 60 weeks of age, all mice with hydronephrosis exhibited chronic eosinophilic inflammation and adenomas that progressed to adenocarcinomas with high CLP expression.

Conclusion: Inflammation and epithelial proliferation at the ureteropelvic junction began with a single infiltration of eosinophils at the juvenile stage in mice. Eosinophilic inflammation is associated with the development of hydronephrosis and urothelial hyperplasia, which may progress to cancer due to increased CLP expression.

35: ALCIAN BLUE INFUSION AS A TOOL FOR GLYCOCALYX VISUALIZATION IN THE HORSE DISTAL LIMB

Cassandra Powers, Abigail Cox, Sandra Taylor, Timothy Lescun, Daniel Hogan, Hsin-Yi Weng, Masahiro Murakami, Ann Weil, Allen Page
Purdue University, West Lafayette, IN, USA

Background: Laminitis is a debilitating and life-threatening disease in horses that affects the epidermal and dermal tissues of the hoof. Glycocalyx, a gel-like layer covering the luminal surface of vascular endothelial cells, is essential for maintaining homeostasis in the vasculature; little is known about the glycocalyx due to the difficulty in identifying it microscopically.

Objective: This study's objective is to test the methodology of Computed Topography (CT) guided perfusion of Alcian blue to enhance visualization of the microscopic glycocalyx. Results of this study will be used to evaluate the use of clopidogrel infusions to mitigate clinical signs in horses with experimentally induced laminitis.

Methods: Using CT, the digital vasculature of the left forelimb of four adult horses were infused with Alcian blue dye. Animals were euthanized shortly after perfusion. Both forelimbs were disarticulated at the metacarpophalangeal joints and hooves were sectioned. Ten samples of lamina are collected from each hoof post-infusion. Three samples are submitted for histopathological evaluation, three samples are submitted for scanning electron microscopy, and four samples are used for mechanical tension testing.

Results: Upon gross evaluation of the limbs from the 4 horses used for perfusion, 1/4 showed strong diffuse perfusion of the Alcian blue, 1/4 showed weak multifocal perfusion, and 2/4 showed no evidence of perfusion. Histopathologic and ultrastructural evaluation is ongoing.

Conclusions: The cumbersome methodology of perfusing alcian blue into the vasculature of the distal horse limb has little value in the outcome of the visualization of the histopathology and ultrastructure of the hoof.

36: IMMUNIZATION WITH MVA-BASED VACCINATIONS PROTECTS K18-HACE2 MICE FROM SARS-COV-2 INFECTION ASSOCIATED LESIONS IN BRAINS AND EYES

Malgorzata Alicja Rosiak[1,2], Georg Beythien[1,2], Lukas Mathias Michaely[1], Sabrina Clever[1], Sandra Lockow[1], Malgorzata Ciurkiewicz[1], Lisa Allnoch[1], Kathrin Becker[1], Christian Meyer zu Natrup[1], Tamara Tuchel[1], Alina Tscherne[3], Leonard Limpinsel[3], Gerd Sutter[3], Asisa Volz[1], Wolfgang Baumgärtner[1], Kirsten Hülskötter*[1], Katharina Manuela Gregor*[1]
[1]University of Veterinary Medicine, Foundation, Hannover, Germany, [2]Center for Systems Neuroscience, Hannover, Germany, [3]Ludwig Maximilian University of Munich, Munich, Germany

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the etiological agent of coronavirus disease-2019 (COVID-19). K18-hACE2 mice, a well-established COVID-19 model, develop severe disease with pneumonia, neuroinvasion and high mortality upon infection. Vaccinations based on the modified Vaccinia Virus Ankara (MVA) provide protection against SARS-CoV-2 in multiple animal models.

Objective: The aim of this study is to evaluate the neuroprotective properties of different MVA vaccines and immunization protocols against SARS-CoV-2 infection in K18-hACE2 mice in the central nervous system (CNS).

Methods: K18-hACE2 mice were infected with SARS-CoV-2 or received PBS. In the following vaccination experiment, PBS, vector, recombinant MVA expressing native (S) or stabilized (St)

SARS-CoV-2 spike protein, nucleocapsid protein (N) or both St and N proteins were administered twice (day 0 and 21), followed by infection with SARS-CoV-2 four weeks later. In an additional experiments, mice were immunized only once and infected two days or four weeks later. Eyes and brains were evaluated histologically and immunohistochemically.

Results: Virus antigen-positive neurons and mild degenerative changes were detected in retinas of unvaccinated infected animals. Brains showed mild to moderate, lymphohistiocytic inflammation, microgliosis and numerous virus antigen-positive neurons. Groups immunized four weeks prior to infection with vaccines containing viral spike protein, lacked or displayed only minimal inflammatory changes with no neuroinvasion. Animals infected two days after immunization, showed mild lesions compared to unvaccinated control groups.

Conclusion: Animals immunized once or twice with SARS-CoV-2 spike protein containing vaccines four weeks prior to infection, were protected from inflammatory lesions and invasion of the CNS.

37: USING NOVEL RIPK3 AND MLKL KNOCK-IN MOUSE MODELS TO STUDY THE ROLE OF NECROPTOSIS IN CHRONIC LIVER DISEASE AND LIVER CANCER

Ramasamy Selvarani[1], Hoang Van Michelle Nguyen[2], Sunho Lee[1], Natesan Pazanivel[3], Raman Muthsamy[4], Roman Wolf[5], Sathyaseelan Deepa[1], Arlan Richardson[1,5]
[1]Department of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma city, OK, USA, [2]Department of Nutritional Sciences, University of Oklahoma Health Sciences Centre, Oklahoma City, OK, USA, [3]Department of Veterinary Pathology, TANUVAS, Chennai, India, [4]Saveetha Institute of Medical &Tech Sciences, Chennai, India, [5]Oklahoma Veteran Affairs Medical Center, Oklahoma City, OK, USA

Background: Non-resolving, chronic inflammation arising from obesity is an important driver of chronic liver disease (CLD) and liver cancer.

Objective: The goal of this study was to determine if necroptosis, which is a programed/regulated cell death pathway that leads to chronic inflammation, plays a role CLD and liver cancer in mice fed a western diet (WD).

Methods: Necroptosis was induced in liver using two knockin (KI) mouse models that overexpress genes involved in necroptosis (Ripk3 or Mlkl) specifically in liver (i.e., *hRipk3-KI* and *hMlkl-KI* mice). These mice and control mice (not overexpressing Ripk3 or Mlkl) were fed a WD (high in fat, sucrose, and cholesterol) starting at 2-months of age for 3-, 6-, and 12-months.

Results: Feeding the WD induced necroptosis in the control mice, which was further elevated in the *hRipk3-KI* and *hMlkl-KI* mice and was associated with a significant increase in inflammation in the livers of the *hRipk3-KI* and *hMlkl-KI* mice compared to control fed the WD. Overexpressing Ripk3 or Mlkl significantly increased steatosis and fibrosis compared to control fed the WD. Mice fed the WD for 12-months developed liver tumors (hepatocellular adenomas): 28% of the control mice developing tumors compared to 62% of the *hRipk3-KI* and *hMlkl-KI* mice. The *hRipk3-KI* and *hMlkl-KI* mice showed significantly more and larger tumor nodules.

Conclusions: Our study provides the first direct evidence that necroptosis arising from hepatocytes can lead to the sequential progression of hepatic steatosis to fibrosis in obese mice that eventually results in an increased incidence in hepatocellular adenomas.

38: PORCINE ASTROVIRUS 4 AS A CAUSE OF TRACHEITIS AND BRONCHITIS IN PIGS

Jazz Stephens[1], Alexa Buckley[2], Rachel Derscheid[3], Rebecca Dubois[4], Danielle Haley[4], Panchan Sitthicharoenchai[1], Calvin Ko[3], Andrew Noel[3], Phil Gauger[3], Jennifer Groeltz-Thrush[3], Michael Rahe[1]

[1]North Carolina State University, Department of Population Health and Pathobiology, Raleigh, NC, USA, [2]Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA, USA, [3]Iowa State University, College of Veterinary Medicine, Department of Veterinary Diagnostic and Production Animal Medicine, Ames, IA, USA, [4]University of California Santa Cruz, Department of Biomolecular Engineering, Santa Cruz, CA, USA

Astroviruses are single-stranded RNA viruses shown to be associated with gastroenteric and neurologic disease in several animal species, including humans. Porcine astrovirus 4 (PoAstV4) has previously been associated with clinical respiratory disease and lesions of tracheitis and bronchitis in pigs; however, experimental reproduction of respiratory disease has not been performed. The objective of this study was to fulfill Koch's postulates by inoculating four-week-old cesarean-derived colostrum-deprived piglets (n=18 challenged and n=11 negative controls) with a PoAstV4 positive tissue homogenate through both intratracheal and intranasal routes and subsequently evaluating pigs for respiratory tract infection, gross and microscopic lesions, and seroconversion. PoAstV4 PCR results on nasal swabs showed a robust infection with peak shedding detected at 6 days post-challenge (6DPC). Seroconversion against PoAstV4 was achieved, with IgG against the capsid spike protein detected at 14DPC. Histologic scoring of the trachea and bronchi revealed lesions of epitheliotropic viral infection with mononuclear infiltration of the lamina propria in challenged pigs. Infection of the epithelium of the nasal turbinates, tracheas, and bronchi of challenged pigs at 5DPC was confirmed with PoAstV4 *in situ* hybridization (ISH) with quantitative assessment pending. Additionally, the characterization of the lamina propria mononuclear infiltrate with CD3 and CD20 immunohistochemistries is ongoing. These findings show that PoAstV4 is a cause of tracheitis and bronchitis in pigs and subsequent analysis will further characterize both the infection and the host immune response.

39: DIFFERENCES IN SUSCEPTIBILITY TO SARS-COV-2 INFECTION AMONG TRANSGENIC HACE2-HAMSTER FOUNDER LINES

Arnaud Van Wettere[1], Scott Gibson[2], Yanan Liu[2], Rong Li[2], Brett Hurst[2], Zhiqiang Fan[2], Venkatraman Siddharthan[2], Irina Polejaeva[2], Bart Tarbet[1], Zhongde Wang[2]
[1]Department of Veterinary, Clinical, and Life Sciences, College of Veterinary Medicine, Utah State University, Logan, UT, USA, [2]Department of Animal, Diary and Veterinary Sciences, College of Agriculture and Applied Sciences, Utah State University, Logan, UT, USA

Animal models recapitulating the clinical and pathological manifestations of SARS-CoV-2 infection are critical to understanding viral pathogenesis and developing effective vaccines and therapies. The golden Syrian hamster is a valuable model for the study of SARS-CoV-2 respiratory disease since hamsters are susceptible to wild type SARS-CoV-2 without the need for host adaptation. However, infected hamsters resolve infection quickly and develop limited clinical disease. A human angiotensin-converting enzyme 2 (hACE2) transgenic hamster was created to generate a lethal model of SARS-CoV-2 infection. During development of the model, differences in susceptibility to SARS-CoV-2 lethal infection was observed between hACE2 transgenic hamster founder lines. The highly susceptible and uniformly lethal hACE2 founder lines FOF35 and FOM41 rapidly progressed to severe infection and death within approximately 6 days post-infection at all three low infectious doses of $10^{0.9}$, $10^{0.3}$, and $10^{0.15}$ 50% cell culture infectious dose ($CCID_{50}$) evaluated. In contrast to the highly susceptible founder lines, virus challenge with the same infectious doses of animals from the F0M44 and F0M51 founder lines resulted in 0 to 20 % mortality. Clinical signs of infection included lethargy, weight-loss, dyspnea, and mortality. Infected hACE2-hamsters develop rhinitis, tracheitis, bronchointerstitial pneumonia, and encephalitis. Mortality was attributed to encephalitis with possible contributions from the accompanying pneumonia. Our studies demonstrate that hACE2 transgenic hamsters differ in their susceptibility to SARS-CoV-2 infection, based on the transgenic hamster founder lines. Variation in the hACE2 transgene integration site is thought to be responsible for variation in susceptibility to SARS-CoV-2 infection.

40: BATCH-DEPENDENT PHENOTYPIC AND BIOLOGIC DIFFERENCE OF CHEMICALLY-INDUCED TUMORS IN A CLOSED COLONY OF SIBERIAN HAMSTERS (PHODOPUS SUNGORUS)

José Vilches-Moure, Ching-Hsuan Chen, Nathan Fisher, Nirk Quispe-Calla, Norman Ruby
Stanford University School of Medicine, Stanford, CA, USA

Background: N-nitroso-N-methylurea (NMU) is a member of the class N-nitrosureas, and has a role as a carcinogen, mutagen, teratogen, and alkylating agent. NMU has been used for the experimental induction of tumors in various animal models. In the literature, the reported resulting neoplasms are varied, and include mammary, renal, pancreatic, gastrointestinal, hepatic, and nervous system tumors. Most reports in rodent models suggest that administration of NMU commonly yields mammary epithelial tumors, both benign and malignant, when injected intraperitoneally and intravenously.

Methods: Siberian hamsters (*Phodopus sungorus*) from a closed colony were injected with NMU from different vendors, and tumors were evaluated.

Results: NMU batches from different vendors yielded tumors with varying latency periods (e.g. time from injection to gross tumor detection), different phenotypes, and different biologic behaviors. Tumors from the initial cohort are phenotypically consistent with epithelial neoplasms, and include adenomas, carcinomas, and adenocarcinomas. Animals often had multiple epithelial tumors, and tumors are largely well-demarcated and lack evidence of local infiltration into the adjacent tissues. Conversely, tumors recently induced with a different batch of NMU yielded neoplasms with a mesenchymal morphology. These mesenchymal tumors were generally solitary and have extensive local tissue infiltration, inflammatory infiltrates, and rare metastases.

Conclusions: Our results show how the same type of reagent but from different vendors can yield different outcomes, and underscores the importance of using (when possible) reagents from the same manufacturer to ensure reproducibility across experiments.

Industrial and Toxicologic Pathology Posters

41: ENHANCING PESTICIDE SAFETY: INVESTIGATING IMPACTS ON MALE HONEY BEE REPRODUCTIVE HEALTH

Marina Carla Bezerra da Silva[1], Saylor Martian[1], Madison Kindopp[1], Midhun Jose[1], Oleksii Obshta[1], Thanuri L.K. Edirithilake[1], Emilio Enrique Tellarini Prieto[1], M. Fahim Raza[1], Marcelo Camilli[1], Belarmino Eugênio Lopes Neto[1], Julia Tregobov[1], Juan Pablo A. Marmolejo[1], Maria Janser[1], Harmony Woodrow[1], Ryan Merkl[1], Justin Slobodian[1], Adriana Martinez Arias[1], Rosephine Enadeghe[1], Ivanna Kozii[2], Igor Moshynskyy[1], Elemir Simko[1], Sarah Wood[1]
[1]Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada, [2]Prairie Diagnostic Services Inc – University of Saskatchewan., Saskatoon, SK, Canada

Background: The widespread use of pesticides due to agricultural intensification has been associated with chronic pesticide exposure in honeybees. Current pesticide safety testing for pollinators primarily focuses on workers, with limited evaluation of reproductive castes, including male honeybee drones. This study aimed to 1) develop an *in vitro* rearing protocol for drones from larva to adult and 2) evaluate the effects of exposure to a known gonadotoxic pesticide (amitraz) on drone testicular development.

Methods: We developed a protocol to rear drones to adulthood in the laboratory by modifying an existing protocol for honey bee workers, including changing the day of larval transfer to the laboratory, diet volume, pupation plate orientation, and the absorbent tissue within pupal wells. Drone

survival was monitored daily. To develop a model for gonadotoxicity in drones reared *in vitro*, we exposed drone larvae to incremental doses of the pesticide amitraz in the diet and evaluated the effects on testicular development, including testes' weight, histopathology, and distribution of germline and somatic cells by *in-situ* hybridization chain reaction.

Results: We successfully achieved 74% ±3.5 % (SEM) survival of drones from larva to adult using our modified *in vitro* rearing protocol. Evaluation of the effects of chronic amitraz exposure on drone testicular development is in progress.

Conclusion: Taken together, these new methods for laboratory rearing and gonadotoxicity assessment for male bees may be used in future pesticide safety evaluation of reproductive castes of honeybees, which will, in turn, have broader benefits for pollinator health, agricultural productivity, and food security.

42: RETROSPECTIVE OF TOXICOLOGIC AGENTS ISOLATED FROM AUTOPSIES OF COMPANION ANIMALS (2019-2024)

Kali M. Griffith[1], Doris M. Miller[1,2]
[1]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [2]Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Background: Poisoning is a common concern of owners submitting companion animals for forensic autopsy; however, a low percentage of these yield positive results upon toxicologic analysis. The majority of confirmed poisonings are accidental with less than 1% reported to be intentional, including non-malicious incidents. Animals most often involved in malicious poisonings are dogs with medium to large breeds overrepresented and cats.

Objective: To quantify and characterize toxicologic agents isolated from companion animals submitted to the AVDL autopsy service from January 2019 to June 2024.

Methods: AVDL records were searched for canine and feline autopsies submitted between 2019-2024 using the keyword "ethylene glycol," diagnosis codes for "toxicosis" and test codes for outside laboratory toxicological analyses.

Results: Of 43 companion animal cases positive for a toxin, 34 (79%) were from dogs and 9 (21%) were from cats. The mean age of affected companion animals was approximately 3.80 years; however, few submissions did not include an age. Rodenticides were confirmed in 9 cases (20%) with 8/9 cases involving dogs (89%). Pesticides were confirmed in 5 cases (20%) with 4/5 cases involving young dogs (80%). 16/45 cases (36%) were positive for veterinary pharmaceuticals including euthanasia drugs, gastrointestinal medications and pre-anesthetic medications. 8 cases (17%) were ethylene glycol (antifreeze) toxicosis diagnosed via histopathology alone, and were evenly split with 4 dogs and 4 cats.

Conclusions: The most frequently isolated toxicologic agents were rodenticides (brodifacoum, bromethalin) followed by euthanasia drugs (pentobarbital, phenytoin), veterinary pharmaceuticals (meloxicam, metronidazole) and pesticides (methocarbamol, nonachlor, dieldrin).

43: HISTOPATHOLOGICAL AND TOXICOLOGICAL INVESTIGATION OF HEPATIC LEAD CONCENTRATION AND HEPATORENAL DAMAGE IN WILD EUROPEAN BADGERS (MELES MELES) IN THE UK: PRELIMINARY RESULTS

Shauna Hilton[1], Davide Corbetta[1], Carlo Bianco[1], Andrea Sartorius[1], Ana Ortiz[2], Malcolm Bennett[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, United Kingdom, [2]VPG Histology, Bristol, United Kingdom

Background

European badgers (*Meles meles*) are omnivorous, have small territories, and are frequently found dead by roadsides making them potentially useful bioindicators for environmental heavy metal pollution.

Objective

The study aims to establish if the histological pattern of hepatorenal damage, and reference ranges of hepatic lead concentration in mammalian tissues could be extrapolated to badgers.

Methods

Necropsy and sample collection was performed in containment on 43 found-dead wild European badgers (Meles meles).

Extrapolated hepatic lead concentration classes were normal (≤1mg/Kg bw dw), high (1-5 mg/Kg bw dm), and toxic (≥5mg/Kg bw dw).

Hepatic and renal histopathology was performed on hematoxylin and eosin using a 4-tier semiquantitative histopathological scoring system to assess the severity and extent of changes (0=<10%, 1=10-40%, 2=40-70%, and 3=>70%).

Hepatic scores comprised vacuolar degeneration, cytoplasmic rarefaction, inflammation, and necrosis. Renal scores comprised tubular atrophy and degeneration, fibrosis, inflammation, and glomerulosclerosis. A combined hepatorenal damage score was calculated by adding hepatic and renal scores.

Results

There was a fair positive correlation between hepatic lead concentrations and hepatorenal damage (r=0.36, p=.019), with significant difference between high (mean=5.27) and normal (mean=3.29) extrapolated lead values (p=.045). Females (mean=5.17) had significantly higher hepatorenal damage than males (mean=3.63, p=.050).

Conclusions

The results suggest badger liver and kidney tissue reaction to hepatic lead concentrations, and value limits for high hepatic lead concentration are similar to other mammalian species.

Females may be more sensitive bioindicators; underlying nutritional or metabolic causes could be considered but are unclear and would benefit from further investigations.

44: PATHOLOGY ASSOCIATED WITH HUMAN CAR T CELL ADMINISTRATION IN IMMUNODEFICIENT MICE: A RETROSPECTIVE REVIEW

Renata Mammone[1], Elinor Willis[2,3], Giovanni Finesso[2], Charles-Antoine Assenmacher[2], Alessandra Piersigilli[1,4], Enrico Radaelli[2], Ileana Miranda[1]
[1]Laboratory of Comparative Pathology, Memorial Sloan Kettering Center, Weill Cornell Medicine, and The Rockefeller University, New York, NY, USA, [2]Comparative Pathology Core, University of Pennsylvania, Philadelphia, PA, USA, [3]Altasciences Preclinical, Seattle, WA, USA, [4]Department of Drug Safety Research and Evaluation, Takeda Development Center Americas, Cambridge, MA, USA

Background: Chimeric antigen receptor (CAR) therapy has been a promising treatment for neoplasia and autoimmune disease. Immunocompromised mice are a common model to test efficacy and safety of CAR T cells of human origin. Preclinical toxicity associated with CAR T cell products encompasses a spectrum of morphologic changes with currently limited literature documentation.

Objective: The purpose of this retrospective study is to review the histopathologic lesions associated with human CAR T cell toxicity in immunodeficient (NSG, NOG-EXL) mice submitted to two different institutions. The hypothesized pathogenesis and translatability of the toxicity for each condition are discussed.

Methods: Pooled data across 2 institutions comprising immunodeficient mice (n = 259) administered human CAR T cells submitted from 2017-present is further categorized.

Results: Lesions were categorized into xenogeneic graft-versus-host disease (xGvHD) (n = 130), aberrant proliferation of human T cells with tissue invasion (n = 65), immune effector cell-associated HLH-like syndrome (IEC-HS) (n = 31), vascular disturbances (n = 41), on target off tumor (OTOT) toxicity (n = 7), acute CAR T cell lysis syndrome (n = 2), and CNS necrosis (n = 2).

Conclusions: The present review provides veterinary pathologists with descriptive guidance on the morphology of different preclinical toxicities associated with human CAR T cell therapy in immunodeficient mice. Additional molecular data and detailed information related to each construct are necessary to further investigate translatability of such liabilities to a clinical setting.

45: FYN-TAU INTERACTION PROMOTES ORGANOPHOSPHATE-INDUCED NEUROTOXICITY IN THE RAT MODEL

Nikhil Sanjay Rao, Marson Putra, Christina Meyer, Madison Van Otterloo, Thimmasettappa Thippeswamy
Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Exposure to organophosphates (OP) induces status epilepticus (SE) through cholinergic hyperstimulation. Neuroinflammation and neurodegeneration that follows lead to the development of epilepsy and associated neurotoxic comorbidities. Current antiseizure drugs target seizures but do not prevent or modify epileptogenesis. The Fyn kinase and tau have been implicated in mediating the pathophysiology of neuronal hyperexcitability and neurodegeneration in Alzheimer's disease and epilepsy, and a disruptor of Fyn-tau interaction, saracatinib, rescued neurodegeneration in the kainate rat model. In this study, we investigated the role of Fyn-tau interaction and downstream signaling pathways in mediating diisopropylfluorophosphate (DFP)-induced neurodegeneration in the rat model. Immunohistochemistry of brain sections of DFP-exposed rats confirmed neurodegeneration and neuroinflammation. We found a significant increase in Fyn-tau association in proximity ligation assay in the hippocampus and piriform cortex of DFP-exposed rats at eight days post-SE compared to controls. In the hippocampal post-synaptic density (PSD) fraction, there was a significant decrease of PSD95 at 24 hours and eight days post-SE and significant increases in nNOS, pNR2B, NR2B, pSFK, Fyn and c-Src compared to controls at eight days post-SE. Coimmunoprecipitation of the hippocampal PSD fraction with Tau pulldown showed relative increases in NR2B-Tau, Fyn-Tau and PSD95-Tau interactions at both time-points. There was a significant increase in pSFK in the piriform cortex with relative increases of pNR2B and nNOS at eight days post-SE compared to controls.

These findings indicate that Fyn-tau interaction is a mediator of organophosphate-induced neurotoxicity and a potential therapeutic target for SE-induced neurodegeneration.

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46: METHOD DEVELOPMENT OF A FLOW CYTOMETRY ASSAY FOR ASSESSMENT OF CYNOMOLGUS MACAQUE (MACACA FASCICULARIS) BONE MARROW IN PRECLINICAL STUDIES

Allison Rogich[1,2], Jordon Cole[1], Giselle Borges e Soares[1], David Ahrens[1], Kimberlyn Petty[1], Stephen Berridge[1], Joshua Schipper[1], Abby Schaffer[1], Taya DeVine[1], Jessica St. Charles[1], Kevin Goncalves[3]
[1]Charles River Laboratories, Mattawan, MI, USA, [2]Michigan State University, East Lansing, MI, USA, [3]Deerfield Discovery and Development, Deerfield Management Company, New York, NY, USA

Introduction: Non-human primates (NHPs) serve as an imperative preclinical model in drug development and safety assessment. New methods must be developed to appropriately assess biologically important test article effects.

Objective: To develop a flow cytometry method to assess bone marrow from NHPs. Pilot sample stability data was also generated.

Experimental Design: Fresh bone marrow was collected from the femurs of NHPs following humane euthanasia. A subset of samples was cryopreserved. Cell counts and viability were carried out by Cellaca MX. Briefly, after processing, cells were incubated with monoclonal antibodies against CD45, CD3, CD20, CD21, CD27, and CD138. Viability dye, FVS 575V, was included. Analysis was carried out by a BD LSRFortessa cytometer and BD FACSDiva software. Antibody titrations, fluorescence minus one, and precision assessments were performed. Sample stability involved cell counts and viability on samples stored in RPMI with 10% FBS +/- Streck Cell Preservative at 4ºC over 7 days.

Results: Fresh samples had a median viability 94% (range 78-97%) and median cell count of 6.72 x 10^6 cells/mL (range 1.0-18.74 x 10^6 cells/mL). Cryopreserved samples revealed a median viability 59% (range 50-82%) and median cell count of 1.19 x 10^6 cells/mL (range 0.93-5.6 x 10^6 cells/mL). Intra-assay, inter-assay, inter-instrument, and inter-analyst coefficient of variations for specific cell populations were 0.06-24.15%, 0.82-45.99%, 0.0-64.86%, and 0.03-56.49%. Samples in RPMI with 10% FBS +/- Streck maintained >90% viability for up to ~72 hours.

Conclusion: Flow cytometric assessment of bone marrow collected post-mortem is feasible. Cryopreserved samples are suboptimal for flow cytometric studies.

47: LIPID VEHICLE (MIGLYOL) CAN CAUSE CLINICAL DECLINE AND DOSE-DEPENDENT RESPIRATORY PATHOLOGY

Julia Y Tsai, Mitzi Adler-Wachter, Brendan Schweitzer, Melissa Barker-Haliski, Jonathan Weinstein, Jessica M Snyder
University of Washington, Seattle, WA, USA

Background: Miglyol, a medium chain triglyceride, is a lipid-based vehicle commonly used for delivery of hydrophobic drugs via oral or parenteral routes with few adverse side effects documented following administration in mammals.

Objective: After administration of 100% pharmaceutical grade miglyol intraperitoneally twice daily for 7 days, male C57BL/6 mice developed severe lethargy and respiratory distress with high morbidity and mortality. A pilot study was performed to determine the underlying etiopathogenesis.

Methods: 3-month-old, male C57BL/6 mice received intraperitoneal injections of miglyol twice daily for 7 days at a dose of 4µL/g (n = 10) or 8µL/g (n=10). Control mice (n=4) received 8µL/g of saline. Clinical status was monitored using a scoring system. Mice were euthanized early based on clinical decline or upon experiment completion (day 14) and submitted for histopathology.

Results: All 8µL/g-treated mice died or were euthanized before day 14 whereas all 4µL/g-treated mice completed study without clinical decline. On gross necropsy, mice in both cohorts had clear, fluid-filled masses in the peri-gonadal adipose tissue. Mice in the 8µL/g treatment group had mild to moderate fibrinous adhesions of the cranial abdominal viscera, and the thoracic cavity contained moderate serohemorrhagic pleural effusion with variably severe, multifocal to coalescing pulmonary hemorrhage and congestion. Histologically, mediastinitis, peritonitis, and pulmonary hemorrhage and inflammation were observed. Despite an absence of overt clinical signs, mice in the 4µL/g group showed similar, though milder, pathological findings of the abdominal and thoracic cavities.

Conclusions: Intraperitoneal migylol administration in mice can cause severe, dose-related hemorrhagic lung pathology with peritonitis.

48: TOXICOPATHOLOGICAL EFFECTS OF INFLUENZA MONOCLONAL ANTIBODIES WITH VARIABLE FC EFFECT IN A LETHAL MURINE CHALLENGE MODEL

Rachael Wolters[1,2], Katherine Gibson-Corley[1], James Crowe[1]
[1]Vanderbilt University Medical Center, Nashville, TN, USA, [2]Oregon Health & Science University, Oregon National Primate Research Center, Beaverton, OR, USA

Influenza causes severe morbidity and mortality globally. Despite approved vaccines and therapeutics, complete protection has not been achieved. Monoclonal antibodies (mAbs) offer potential as prophylactic and therapeutic drugs for respiratory viruses, but the role of Fc-mediated effects, how Fc can lead to viral clearance and pathology needs further exploration. To evaluate the role of Fc in therapeutic candidate Influenza type B Virus (IBV) monoclonal antibodies and understand the relationship between protection, viral clearance, and pathology. Six- to eight-week-old female BALB/c mice were administered a 10 mg/kg dose of mAbs, with and without a mutation to abrogate Fc function (LALA PG), 24 hours before a lethal inoculum of IBV. Lungs were collected at 3- and 6-days post-inoculation for viral titering, in situ hybridization (ISH), and H&E staining. A separate cohort of mice was followed in a survival study. Of the three monoclonal antibodies studied all provided complete protection at 10 mg/kg. The antibody with the highest Fc effector function, exhibited the most significant viral clearance and the most severe pathology at day 6 post-infection. Whereas the antibody with the least Fc function, was the most potent in the dose-down study. An inverse relationship between Fc effector function and potency was observed in this prophylactic model. As antiviral therapeutics and biologics continue to expand rapidly, our study highlights the potential for Fc function of monoclonal antibodies to affect their efficacy, underpinning the importance of understanding the interplay and role of Fc functions in drug efficacy and safety warrant further detailed investigation.

49: 1-TRICHLOROMETHYL-1,2,3,4-TETRAHYDRO-BETA-CARBOLINE (TACLO)-INDUCED NEUROTOXICITY FOLLOWING EARLY-LIFE EXPOSURE IN THE ZEBRAFISH MODEL

Ji-Hang Yin, Katharine Horzmann
Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Background: 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) is an endogenous neurotoxicant formed in the brain after exposure to trichloroethylene (TCE) and tetrachloroethylene (PERC). TCE and PERC are industrial solvents and have the potential to induce developmental toxicity, neurotoxicity, and the development of Parkinson’s disease; however, it is unknown if their metabolite, TaClo, triggers similar effects. **Objective:** This study tested the hypothesis that embryonic

zebrafish exposure to environmentally relevant concentrations of TaClo induces developmental toxicity and neurotoxicity. **Experimental design:** Fertilized embryos were dosed with embryo water (control), DMSO (carrier control), TaClo at 5 ppb, 50 ppb, and 500 ppb, and MPTP (positive control) for 24 or 120 hours post fertilization (hpf). Endpoints for developmental toxicity evaluation include survival and hatching percentage, body measurement, and heart rate analysis. Photomotor and visual motor behavioral response tests, and assessment of relative dopaminergic neuronal numbers in the ventral diencephalon using whole mount in-situ hybridization and immunofluorescence assay were used to assess neurotoxicity. Superoxide dismutase and caspase 3 activity were evaluated to investigate the underlying mechanism of TaClo-induced neurotoxicity. **Results:** Our results demonstrated a bimodal dose-response with TaClo at 5 ppb triggering significantly altered larval neurobehavior at 24 and 120 hpf, decreased relative dopaminergic neuronal expression and increased the apoptotic signals in the ventral diencephalon of the larval brain. No significant superoxide dismutase enzymatic activity was noted among the treated groups; however, TaClo at 5 ppb induced a significant apoptotic signal. **Conclusion:** Environmentally relevant concentrations of TaClo induced neurotoxicity following an early-life exposure in the zebrafish model.

Natural Disease Posters

50: FATAL SYSTEMIC SARCOCYSTIS FALCATULA INFECTION IN AN ECLECTUS PARROT (ECLECTUS RORATUS) WITH SECONDARY ESCHERICHIA COLI SEPTICEMIA

Hernando Acevedo[1], Anibal Armien[2], Heather Fritz[2], Eileen Henderson[1], Javier Asin[1], Francisco Uzal[1], Akinyi Nyaoke[1]
[1]California Animal Health and Food Safety (CAHFS) Laboratory System, San Bernardino Branch, University of California-Davis, San Bernardino, CA, USA, [2]California Animal Health and Food Safety (CAHFS) Laboratory System, Davis Branch, University of California-Davis, Davis, CA, USA

A 7-year-old, male, Eclectus parrot [*Eclectus roratus]* was submitted to the California Animal Health and Food Safety, San Bernardino laboratory for necropsy with a 3-day history of lethargy that progressed to bilateral knuckling. On postmortem examination, most skeletal muscles had numerous white, ~0.2 x 0.1 cm oval foci. Both ankle joints contained ~0.1 ml of dense green material. On histopathology, expanding the sarcoplasm of numerous skeletal and myocardial myofibers were protozoan cysts measuring ~20 x 12 um to ~300 x 250 um characterized by an ~ 4 um eosinophilic wall containing myriad ~ 0.1um elliptical zoites. Electron microscopy of the cyst wall showed protrusions with a jagged border and finger-like tip ends, and merozoites. The latter were characterized by micronemes, a central round nucleus, and anterior conoid; rhoptries were not observed. IHC for *Sarcocystis* spp. was positive in brain and skeletal muscle. DNA sequencing of a 462 base pair region of the 28s large subunit rRNA gene identified the organism as most closely related to *Sarcocystis falcatula.* Large numbers of *Escherichia coli* were isolated from the liver, lung, and joint fluid, suggesting a secondary bacterial septicemia. *Sarcocystis* spp*.* are apicomplexan protozoa and cause infection by intracellular proliferation and cyst formation. The disease may be fatal. This case represents a novel presentation of systemic sarcocystosis with secondary *E. coli* septicemia in an Eclectus parrot*.*

51: HERPES VIRUS OCCURRENCE IN CAUCASIAN PIT VIPER AND MACROVIPERA LEBETINA

Hasti Azarabad[1], Ali Taheri[2], Kimia Berenji[1], Yasin Dibavand[1]
[1]Department of Pathology, Hamidreza Fattahian pet hospital, Tehran, Islamic Republic of Iran, [2]Department of Venomous Snake Breeding Center, Faculty of Environment, Tehran, Islamic Republic of Iran

Herpesviruses are large, enveloped viruses that have a double-stranded DNA genome and an icosahedral capsid. The Herpesviridae family comprises a variety of viruses that infect vertebrate hosts. Reptilian herpesviruses have not yet been assigned to any existing genera within the

Herpesviridae family. In reptiles herpesvirus infections can lead to conditions like; tumors, encephalitis, conjunctivitis, stomatitis, hepatitis and death. The only pit viper species occurring in Iran is the Caucasian pitviper Gloydius caucasicus. The genus Macrovipera that is also known as the giant Palearctic vipers, constitutes three species; M. schweizeri, M. razii, and M. lebetina. M. lebetina is widely distributed from Eastern Europe to Central Asia and the Middle East. During the daily visit to the venomous snake breeding center of the faculty of environment, these snakes were found dead. Two captive Caucasian pit viper and Macrovipera lebetina were submitted for postmortem examination. Histological examination on hepatic tissue in Caucasian pit viper revealed multifocal infiltration of lymphocytic cells with heptocyte necrosis. There were numerous intranuclear inclusion body of herpes virus in hepatocytes. Histological examination in Macrovipera lebetina on hepatic tissue revealed multifocal infiltration of lymphocytic cells with heptocyte necrosis. There were numerous intranuclear inclusion body of herpes virus in hepatocytes. Myocardial tissue showed focal infiltration of mononuclear inflammatory cells with moderate myocyte degeneration. Viruses like herpes virus infections can be a serious threat for wildlife species and diagnosing them can be helpful to reduce the risk of disease transmission.

52: HISTOPATHOLOGICAL AND EPIDEMIOLOGICAL STUDY OF ORAL OSTEOMYELITIS IN OVINE POPULATION ON ST. KITTS

Diana Bochynska, Hallie Braeuner, Pompei Bolfa
Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

Introduction: Oral osteomyelitis (OM) affects sheep ability to prehend and masticate food. This study aims to characterize the prevalence, histopathological features, and potential causes of OM in a population of sheep on St. Kitts over a nine-year period.

Materials and Methods: A retrospective review of 146 ovine autopsies from January 2015 to October 2023 was conducted. Data included age, sex, body condition, and presence of oral OM. Affected bones and teeth were identified, and tissue specimens underwent histopathological examination. Bacterial cultures were analyzed to identify pathogens.

Results: 23% of sheep exhibited signs of OM on autopsy. Affected sheep were predominantly 1-5 years old (85%) male (97%). The majority of deaths (55%) occurred in the dry season (March-June). The right mandible was the most commonly affected (25 cases), with the molars being the most frequently involved teeth (39%). Gross findings included asymmetrical dental attrition, wave mouth, missing teeth, empty sockets, and tooth root abscesses. Histological findings included variable degrees of lymphoplasmacytic and neutrophilic, necrotizing osteomyelitis, with fibrosis and granulation tissue formation. *Pseudomonas aeruginosa* was the most commonly isolated bacterium.

Conclusion: OM in sheep on St. Kitts is strongly correlated with emaciation and poor body condition. Younger male sheep are more commonly affected, possibly due to behavioral factors. The dry season appears to exacerbate the incidence of OM, potentially due to mechanical injuries from dry forage. This study underscores the need for better husbandry practices to reduce OM and improve sheep welfare in St. Kitts and similar climates.

53: INTESTINAL CARCINOMA IN HORSES: MORPHOLOGIC AND IMMUNOLOGIC ANALYSIS

Laura K Bryan
Texas A&M University, College Station, TX, USA

Eleven horses presenting to a tertiary veterinary hospital between 2003 and 2023 were diagnosed with small and large intestinal carcinomas. Average age at time of diagnosis through biopsy or necropsy was 17 years ± 7.2 years (range 3 – 28 years), with colic being the most common reason for patient admission. The most common breed-sex combination was Arabian mares and Quarter

Horse geldings. Jejunum was the most common site, often with multiple masses (average 7.6 cm diameter). Two horses had tumors develop at the ileocecal junction, two at the dorsal large colon, and one in the ventral large colon. Osseous metaplasia, stenosis of the intestinal lumen, and ulceration at tumor sites were common. Most tumors were locally invasive with a few cases demonstrating metastasis to distant sites. SOX9 immunohistochemistry was variable between the neoplasms.

54: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MYCOPLASMA BOVIS MASTITIS IN DAIRY COWS

Fernanda Castillo-Alcala[1], Leah Cristina Gans[2], Kevin Patrick Kearney[3]
[1]Tāwharau Ora – School of Veterinary Science, Massey University, Palmerston North, New Zealand, [2]Awanui Veterinary, Palmerston North, New Zealand, [3]Veterinary Centre, Oamaru, New Zealand

Background: *Mycoplasma bovis* causes several disease syndromes including mastitis and arthritis in adult cattle, and pneumonia, arthritis, and otitis media in calves. Histopathological lesions associated with *M. bovis* pneumonia and arthritis have been well-described in both experimental and natural infections. However, the lesions associated with *M. bovis* mastitis in natural infections are rarely reported.

Objectives: To characterize the histopathological lesions of *M. bovis* mastitis in a natural disease outbreak and confirm the presence of *M. bovis* using immunohistochemistry.

Methods: Mammary tissue samples were obtained at the time of slaughter from 14 cows diagnosed with *M. bovis* clinical mastitis (confirmed via *M. bovis* milk qPCR). Gross examination of the mammary glands was conducted at the time of slaughter, and samples of all quarters were submitted for histological examination and immunohistochemistry.

Results: The most common histological lesions were interstitial and alveolar inflammation (98.2% and 92.7%, respectively), alveolar atrophy (90.9%), and fibrosis (90.9%). The morphology of inflammatory lesions varied amongst individual quarters with the most common pattern being granulomatous or pyogranulomatous (47.3%) followed by neutrophilic (27.3%), lymphocytic and plasmacytic (14.5%), mixed (3.6%) or no inflammation (7.3%). Areas of caseous necrosis were present in 41.8% of the quarters examined. Of the 55 quarters examined, 54.5% were immunoreactive for *M. bovis*, and 3.6 % yielded a “suspicious” result. Strong immunoreactivity for *M. bovis* was most identified within the foci of caseous necrosis.

Conclusions: Our findings suggest a wider variation in the histological lesions associated with *M. bovis* mastitis in natural infections than those originally reported.

55: ASSESSING THE HEALTH OF INVASIVE TRACHEMYS SCRIPTA: INSIGHTS FROM A CONTROLLED CONTAINMENT CENTER IN NORTHEAST ITALY

Luca Spadotto, Niccolò Zanirato, Cinzia Centelleghe, Sandro Mazzariol, Laura Cavicchioli
Dept of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy

Trachemys scripta is considered among the most Invasive Alien Species (IAS) worldwide. The European Union applies a series of restrictions to limit their spreading into Unional territories. For these reasons, University of Padua, Italy, according to the regional administration, established in 2022 a containment center designed for American pond-sliders found in the wild or transferred by citizens. Inside this center, located in “Parco Regionale Delta del Po” in Northeast Italy, 108 individuals live under the care of staff members trained on IAS. Before entering the facility, each animal undergoes a health check through microbiological oropharyngeal and cloacal swabs. If Salmonella is detected, the animal must be humanely euthanized, since Salmonella is a potential threat to public

health and safety. Since 2022, twenty-nine animals have been found dead, with seventeen individuals subjected to a complete post-mortem procedure. After the necroscopic examination, samples were collected for histopathology and virology.
Microbiological results revealed that none of the turtles tested positive for Salmonella. The most significant pathogens found in the cloaca are *Aeromonas hydrofila* and *Pantotea agglomerans*, in the oropharynx are *Aeromonas hydrofila* and *Erwinia persicina*. Moreover, the presence of Herpesvirus has been investigated, however, the results are still pending.
Pathological findings observed during the necropsy were hepatic lipidosis and pulmonary edema, with one individual presenting oviductal impaction.
As previously noted, American pond-sliders pose a potential risk to human and animal health. Therefore, ongoing epidemiological monitoring is essential to prevent the emergence and spread of infectious diseases in local European fauna.

56: REVIEWING THE RECORDS: SPONTANEOUS AMYLOIDOSIS IN WILD RABBITS (SYLVILAGUS SPP.); 1984-2024

Elliott Chiu[1,2,3], Arely Rosas Rosas[1]
[1]San Diego Zoo Wildlife Alliance, San Diego, CA, USA, [2]University of California, Davis, Davis, CA, USA, [3]City of University, Hong Kong, Hong Kong, Hong Kong

Current literature regarding spontaneous amyloidosis in rabbits undercuts the significance of this disease in rabbits. While it is by no means a novel disease, amyloidosis in rabbits exists primarily in the context of inducible disease with the most common manifestation as renal deposition. A previous survey of renal disease in rabbits identified a low prevalence (2 out of 312; 0.6%) leading others to conclude and report that amyloidosis is not a significant disease in rabbits. Here, we examine the presence and distribution of amyloid in wild rabbits (*Sylvilagus* spp.) including desert cottontails and brush rabbits found dead or euthanized on grounds of the San Diego Zoo Safari Park. Between August 1984 and April 2024, 157 rabbits were accessioned and full gross necropsies and histology was performed. Out of these cases, 29 rabbits were diagnosed with renal or systemic amyloidosis. Renal (interstitium, glomeruli, or both) amyloidosis was the most common manifestation. Vessels were the second most affected organ disseminated throughout numerous organs. The spleen, heart, stomach, and adrenal gland were consistently but less affected, and sometimes inconspicuously so without the aid of Congo Red staining. Internal parasitism with cestodes and to a lesser degree nematodes, but not coccidia, were significantly correlated with amyloidosis. Our results provide a stark contrast to previous reports of amyloid in rabbits demonstrating that not only is spontaneous amyloidosis relatively common, but should be considered as a potential cause of death in wild rabbits.

57: VARIATIONS OF LIVER HISTOPATHOLOGY AND CLINICAL DIAGNOSTICS WITH COMMON BILE DUCT OBSTRUCTION IN 20 DOGS AND CATS

Eunju (April) Choi, Tarini Ullal
University of California-Davis, Davis, CA, USA

Background: The histopathologic and clinical spectrum of obstructive cholangiopathy in dogs and cats need to be better defined.

Objective: To describe features of obstructive cholangiopathy in dogs and cats with an obvious cause of obstruction.

Methods: The University of California-Davis, Veterinary Medical Teaching Hospital pathology database was searched from 2000-2024 for the term “common bile duct mass” and the ultrasound database was searched for “EHBDO” for extrahepatic bile duct obstruction. Cases with liver histopathology, ultrasound report, and clinical chemistry were identified.

Results: Twelve cats and 8 dogs were identified. Eleven were biopsies and 9 were necropsies. The primary cause of mass effect identified from pathology was malignant tumors (10 cats, 3 dogs). Ultrasound reports mentioned EHBDO with liths (2 cats, 1 dog) and pancreatitis (3 dogs). All animals but a cat with lymphoma had ultrasonographic features of obstruction including dilation and/or mural thickening of the duct system. Total bilirubin was increased in all cases but a cat with cholangiocarcinoma and a dog with lymphoma. Canalicular cholestasis was seen in 2 of 12 cats and 4 of 8 dogs. Common large bile duct histologic features in cats were moderate to severe ectasia and peribiliary edema (8/12) whereas dogs had mild to moderate ectasia and peribiliary fibrosis (6/8). Three biopsies did not have large ducts to examine. Apart from one dog and cat, the smallest portal tracts were replaced by ductular reaction.

Conclusions: This small cohort of obstructive cholangiopathy demonstrates the variation of liver histopathology and clinical presentation in dogs and cats.

58: BLOOD SERUM DERIVED EXTRACELLULAR VESICLES AS A POTENTIAL MECHANISM OF CHRONIC WASTING DISEASE PERIPHERALIZATION IN WHITE TAILED DEER

Jesse Cole, Erin McNulty, Amy Nalls, Joseph Westrich, Candace Mathiason
Prion Research Center, Colorado State University, Fort Collins, CO, USA

Background:

Chronic Wasting Disease (CWD) is a rapidly spreading, fatal neurodegenerative prion disease of cervid species that is known for its unprecedented facile transmission. The infectious agent that causes disease has been detected in the blood of prion-infected hosts. As CWD prions traffic across mucosal surfaces into the blood stream within minutes post oral consumption, there is increased need to determine the role blood-borne prions play in disease initiation and pathogenesis.

Objective:

We aim to elucidate the relationship between extracellular vesicles (EVs) and mechanisms of CWD peripheralization. We hypothesize that EVs facilitate hematogenous dissemination of prions, contributing to the dispersal of the infectious agent to tissues with capacity to support disease initiation and progression.

Methods:

Differential centrifugation, size exclusion chromatography (SEC), immunoblotting, transmission electron microscopy (TEM), bicinchoninic acid assay (BCA), and nanoparticle tracking analysis (NTA) were performed to isolate, characterize, and quantify blood serum derived EVs. Real-time quaking-induced conversion (RT-QuIC) and serial protein misfolding cyclic amplification (sPMCA) were performed to assess for the presence of prion seeding activity (prions) in EV samples.

Results:

Blood serum derived EVs have been harvested from naïve and CWD-infected white-tailed deer. Analysis for the presence of prion seeding (prions) within EV samples is ongoing.

Conclusions:

This, to our knowledge, demonstrates the first characterization of EVs in a cervid model. Our findings suggest EV isolates exhibit a concentration dependent inhibitory effect on prion seeding detection. Thus, modification of prion seeding assays is ongoing to further refine their ability to assess prion burden in cervid EV samples.

59: EVALUATION OF DIAGNOSTIC AGREEMENT BETWEEN CRYOSECTION AND FORMALIN-FIXED PARAFFIN-EMBEDDED HEMATOXYLIN AND EOSIN-STAINED SLIDES IN CENTRAL NERVOUS SYSTEM MASSES

Elena Alina Demeter[1], Ta-Chung Mou[2], Ashleigh Newman[3], Tracy Stokol[3], Jonathan Woods[4], Yael Merbl[4], Andrew Miller[1]
[1]Department of Population Medicine and Diagnostic Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [2]Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [3]Department of Population Medicine and Diagnostic Sciences, Section of Clinical Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY, USA, [4]Department of Clinical Sciences, Section of Neurology and Neurosurgery, Cornell University College of Veterinary Medicine, Ithaca, NY, USA

Background: Hematoxylin and eosin (H&E) stained cryosections offer a rapid diagnosis that can guide intraoperative clinical decisions and treatment and provide margin evaluation. The degree of agreement between cryosections and formalin-fixed paraffin-embedded (FFPE) H&E slides evaluation is unexplored in veterinary species.

Objective: To compare the diagnosis accuracy of H&E cryosections to the gold standard of FFPE H&E slides in canine and feline central nervous system (CNS) space-occupying mass lesions undergoing surgical debulking or excision.

Method: Cases were collected between 2019 and 2024 from cats and dogs with CNS space-occupying masses undergoing debulking/excision at Cornell University Hospital for Animals. Histologic evaluation of cryosections and FFPE H&E slides was performed by randomly assigned board-certified anatomic pathologists. The results were compared for agreement considering diagnosis and, when applicable, tumor subtype, invasion, atypia, mitoses, inflammation, and necrosis.

Results & Conclusions: Nine cases were identified and analyzed: 5 meningiomas, 1 osteosarcoma, 1 plasma cell tumor, 1 oligodendroglioma, and 1 abscess. Diagnosis agreement was achieved in 7 out of 9 cases. One case was non-diagnostic on cryosection. The most common diagnosis was meningioma (5/9), identified correctly by both methods. In 3 out of the 5 meningiomas, the histologic subtype was also in agreement between the methods. The features with the lowest agreement were mitotic activity and degree of atypia. Inflammation and necrosis were consistently not identified by cryosection, regardless of diagnosis. Invasion could not be assessed by cryosection evaluation. In 8 out of 9 cases, patients had improvement of clinical signs; 1 case was euthanized intraoperatively.

60: GROSS AND HISTOPATHOLOGIC LESIONS ASSOCIATED WITH LAMENESS IN GROWER PIGS

Nathan Fanzone, Meghann Pierdon, David Buckwalter, Elisabeth Lemmon, Julie Engiles
University of Pennsylvania, Philadelphia, PA, USA

Background: Lameness is associated with negative welfare and economic outcomes but is understudied and difficult to diagnose in the field. In grower pigs, lameness prevalence has been reported up to 19.7%.

Objective: Evaluate lesions in viscera and appendicular bones and joints from lame and non-lame growing pigs.

Methods: Ten lame pigs and 10 age-matched non-lame control pigs from 10 farms were euthanized and evaluated for gross and histologic lesions in viscera, joints, and sub-sectioned bones. Select control-matched joints from lame pigs were tested for *Mycoplasma hyosynoviae* via PCR.

Results: Lame limbs had 12 times greater odds (95%CI 3.4-48.6) of having a grossly-identified synovial lesion, and 15.7 times greater odds (95%CI 3.8-89.7) of having a grossly-identified bone lesion, compared to control limbs. Gross synovial lesions included effusion and hypertrophy with edema and hyperemia but without overt exudate. Histologically, this correlated to increased vascularity, subintimal fibrosis, and variable amounts of lymphohistiocytic and neutrophilic inflammation. Synovium from one lame pig was PCR-positive for *Mycoplasma hyosynoviae*. Almost all histologic bone lesions were mild-moderate *osteochondrosis latens* and *manifesta*. There was no difference between groups in the odds of having a visceral lesion or severe histologic bone lesion.

Conclusions: Non-suppurative inflammatory synovial lesions were associated with lameness, while visceral or inflammatory bone lesions were not. Histologically, osteochondrosis lesions were evenly distributed across lame and control pigs. Given the polyarticular synovial lesions in most of the lame pigs, infectious etiologies, such as *Mycoplasma hyosynoviae* or other pathogens, cannot be excluded as potential contributing causes of lameness.

61: A HISTOPATHOLOGICAL SURVEY OF ENDOPARASITES IN THE GASTROINTESTINAL AND RESPIRATORY TRACTS OF WILD OPOSSUMS (DIDELPHIS MARSUPIALIS) IN TRINIDAD

Karelma Frontera-Acevedo, Roxanne Charles, Rod Suepaul
University of the West Indies, St. Augustine, Trinidad and Tobago

Background: The black eared opossum (*Didelphis marsupialis*) is an American marsupial native to the island of Trinidad. They are regularly hunted on the island despite the paucity of information of parasites in this species.

Objective: This study is part of a broader research project investigating diseases in various neo-tropical animals in Trinidad, and aims to detect and identify gastrointestinal and respiratory parasites found in wild opossums caught in the island.

Methods: Seventy-one wild opossums were caught between 2014 and 2020. Tissue samples and gastrointestinal contents were collected for histopathological and parasitological analysis respectively.

Results: Parasitological evidence of gastrointestinal infection was observed in 57 (80.3%) cases. Eighteen of those cases (31.6%) were supported by histopathology. However, six cases had histopathologic evidence of gastrointestinal parasitism without parasitological support. Three nematodes were found in the stomach (4.2%), nine parasites (including coccidian) in the small intestine (12.7%), and 15 nematodes in the large intestine (21.1%). The nematodes *Turgida turgida* and *Cruzia tentaculata* were amongst those identified. An acanthocephalan (*Oligacanthorhynchus* spp) was identified in the small intestine. In the respiratory tract, four (5.6%) lungworms were observed, three of them nematodes, and one trematode. Three opossums had evidence of respiratory and gastrointestinal parasitic co-infections.

Conclusion: To the best of our knowledge, this is the first histopathological report of gastrointestinal and respiratory parasites detected in opossums in Trinidad. The parasites found have been identified in the same or similar species of opossums in other regions of the Americas, and none are considered zoonotic.

62: TREPONEME-ASSOCIATED HOOF DISEASE, AN EMERGING DISEASE IN FREE-RANGING ELK (CERVUS CANADENSIS), IS ASSOCIATED WITH A VARIETY OF BACTERIAL OPERATIONAL TAXONOMIC UNITS

Elizabeth Goldsmith[1], Kyle Taylor[1], Devendra Shah[2], Margaret Wild[1]
[1]Washington State University, Pullman, WA, USA, [2]Texas Technical University, Lubbock, TX, USA

Background: Treponeme-associated hoof disease (TAHD) causes lameness and debilitation in free-ranging elk. TAHD lesions are characterized by suppurative and ulcerative pododermatitis with intralesional argyrophilic spirochetes. Apparent disease prevalence varies with heavily afflicted herds in some areas and sporadic cases in other areas. Initial investigations have suggested a polybacterial etiology; however, studies were limited by small sample sizes or small study areas.

Objective: Our study objective was to identify and compare bacterial communities associated with a spectrum of histologic hoof lesions and with elk hooves collected across a broad geographic range.

Methods: We examined tissues from 116 free-ranging elk with grossly normal or abnormal hooves from collection areas with higher TAHD prevalence (western Washington and northwestern California), sporadic cases (central and eastern Washington and northern Idaho), or with no TAHD detected (South Dakota). We performed histopathology to diagnose TAHD and categorize hoof lesions, and we conducted 16S rRNA gene amplicon sequencing to identify microbial communities within hoof tissues. We analyzed 16S data to identify variation in microbial communities across histologic lesion categories and geographic areas.

Results & Conclusions: *Treponema, Mycoplasma, Fusobacterium,* and other bacterial operational taxonomic units (OTUs) were associated with TAHD-positive lesions and with pustular dermatitis and erosive to ulcerative dermatitis categories regardless of TAHD diagnosis. Microbial communities varied by collection area and differed between TAHD-positive hooves from areas of higher and lower apparent TAHD prevalence. Our findings support a polybacterial etiology and identify key OTUs characteristic of TAHD-positive lesions to target for future research and diagnostic test development.

63: NOVEL AVIADENOVIRUS DETECTED DURING A MORTALITY OUTBREAK OF BLACK SKIMMERS, TEXAS, USA

Katia Groch[1], Elena Duran[2], Jacquelyn Grace[3], Josue Diaz Delgado[4], Mathias Martins[4], Laura Bryan[1]
[1]Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Science, Texas A&M University, College Station, TX, USA, [2]Texas A&M Interdepartmental Program in Ecology and Evolutionary Biology, College Station, TX, USA, [3]Department of Ecology and Conservation Biology, Texas A&M University, College Station, TX, USA, [4]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA

Black Skimmers (*Rynchops niger niger*) are sentinel coastal aquatic birds that breed in coastal regions of the United States. Black skimmer populations are declining, and little is known about health and diseases of this species. An outbreak of mortality of juvenile black skimmers occurred in the West Galveston and East Matagorda Bay populations during the 2022 and 2023 breeding seasons. We used necropsy, histopathology, and molecular genetic methods to investigate the cause of death in fourteen chicks. Moderate to severe lymphoid depletion with occasional lymphocytolysis was observed in 13/14 (93%) animals, and amphophilic intranuclear inclusion bodies were observed in the cloacal epithelium in 6/14 (43%) cases. Additionally, the animals had high parasite burden. Sequence-Independent, Single-Primer Amplification (SISPA) random sequencing and viral metagenomics on extracted bursal DNA from two birds revealed novel 2,132 bp and 2,610 bp consensus sequences of the aviadenovirus hexon protein and IVa2 protein, respectively. The sequences shared 68% and 73% nucleotide identity to the closest aviadenovirus sequences available in GenBank. To the best of our knowledge, this is the first report of an Aviadenovirus infection in the genus *Rynchops.* The viral infection associated with lymphoid depletion could have increased susceptibility of the birds to parasitic disease, which ultimately contributed to the mortality outbreak.

64: (WHY?)OMING TOADS: RETROSPECTIVE STUDY OF DISEASE IN CAPTIVE INDIVIDUALS

Bridgette Gunn, Brittany McHale, Elizabeth Howerth
University of Georgia, Athens, GA, USA

Wyoming toads (*Anaxyrus baxteri*) are one of the most critically endangered amphibians in North America. Considered functionally extinct throughout their native range in the Laramie Basin of southeast Wyoming, USA, wild populations are currently reliant on captive breeding colonies. Established from several founding individuals of unknown relatedness, recent work has characterized aspects of immunogenetic and microbiome diversity from captive individuals to better understand disease vulnerability of the species. Though infection with *Batrachochytrium dendrobatidis* has been documented in *ex situ* and native populations, little information details the moiety of diseases that can affect captive populations and, by extension, individuals potentially released into the wild. To complement this growing body of research, cases (N= 27) from captive colonies submitted to the University of Georgia's Infectious Disease Laboratory, Zoo and Exotic Animal Pathology Service, from July 2022 to June 2024 were evaluated to characterize causes of disease. Dermatologic pathology was the most prevalent at the time of death (74.1%), with fungal dermatitis accounting for 55% of affected cases. Among remaining commonly identified conditions across all cases, chronic renal disease was noted in 48.1%, hepatocellular degeneration and pigmented macrophage aggregate hyperplasia in 37%, pneumonia in 29.6%, and leukemia in 18.5%. Consistent with the paucity of reports on this species from the 90's, fungal dermatitis continues to be a major morbidity factor. Further efforts to characterize fungal pathogens with molecular testing in these cases is ongoing and is being coupled with viral genome sequencing to explore potential oncogenic viruses linked to the leukemias.

65: RETROSPECTIVE CHARACTERIZATION OF VON MEYENBURG COMPLEXES IN CATS

Emily King, Sara Mayer, Megan Schreeg
The Ohio State University College of Veterinary Medicine, Columbus, OH, USA

Feline cystic biliary lesions are common, but historical debate over lesion pathogenesis and nomenclature has impeded characterization. Most lesions are considered von Meyenburg complexes (VMCs), a form of hepatic ductal plate malformation currently characterized by discrete fibrotic areas with embedded irregular bile ducts. The overarching goal of this study was to characterize feline VMCs identified at The Ohio State University College of Veterinary Medicine from 2000-2021. Our first aim qualifies nomenclature used for diagnosis of feline cystic biliary masses. Of 67 candidate cases, "biliary cystadenoma" was the most common diagnostic term (65.6%, 44/67) followed by "biliary tree anomaly" (25.3%, 17/67) and "ductal plate malformation" (16.4%, 11/67). The majority (82.1%, 55/67) of evaluated cases were reclassified as VMCs, followed by ductular reaction (7.4%, 5/67) and biliary cyst (5.9%, 4/67). Our second aim characterizes the demographic, gross, histologic and immunohistologic features of feline VMCs. The average age of cats with VMCs was 13.4 YO, with males slightly overrepresented (58.2%, 32/55). Grossly, VMCs were described as focal to multifocal, sub-capsular, multiloculated cystic structures composed of a soft, thin, pale-tan lining and ranging from 2mm – 80mm. Histologically VMCs were characterized by well-demarcated multifocal cysts lined by cuboidal biliary epithelium and surrounded by mild to moderate amounts of fibrous connective tissue with entrapped hepatocytes and minimal lymphoplasmacytic inflammation. Notably, some VMCs (18.1%, 10/55) contained emerging biliary carcinomas, warranting future investigation. Immunohistologic studies investigating proliferation markers in VMCs is pending. Overall, this study establishes the defining nomenclature and pathologic features of feline VMCs.

66: "TAIL NAIL" – MARKED FOCAL CORNIFICATION OF THE TAIL TIP OF RATS

Matthew Lanza, Genesis Newton-Lebron, Danielle Covington
Penn State University College of Medicine, Hershey, PA, USA

BACKGROUND: Tail nail is a colloquial term for large claw- or bone-like lesions that occasionally form at the tail tips of rats. The lesion is described in sporadic reports by owners of pet rats but is largely absent from veterinary literature, leading to clinical misdiagnosis as exposed bone from

trauma, self-mutilation, or other causes of necrosis, resulting in amputation and/or exclusion from experiments. Recognition of the lesion is therefore essential for animal welfare and management.

OBJECTIVE: To describe the gross and histologic features of tail nail, and to discuss etiopathogenesis.

METHODS: From 2022-2024, 4 Sprague-Dawley and 23 Fischer 334 rats representing both healthy controls and experimentally manipulated animals at the Penn State College of Medicine presented for suspected tail injury with exposed bone. 10 lesions were submitted for histopathology. Clinical and experimental data was reviewed for associated factors.

RESULTS: Examinations revealed 2-8mm long, pale tan, firm, conical masses on the tail tips, histologically represented by densely lamellated keratin spires atop hyperplastic squamous epithelium. Adjacent epithelium and subjacent tissues including dermis, blood vessels, and bones were normal. Significant inflammation was not identified. The only associated finding was a history of anesthesia, which may more broadly represent a stressful event.

CONCLUSIONS: Tail nail is a presumably benign accumulation of keratin and is not associated with trauma. Although possibly a disorder of desquamation, lesions are confined to the tail tip with sparing of the limbs and the rest of the tail, unlike ringtail. Stress may be a possible association. Amputation is not indicated.

67: PATHOGENESIS OF INFLUENZA A VIRUS INFECTION IN GREY AND HARBOR SEALS IN THE UNITED KINGDOM

Gail Leeming[1], James Barnett[2], Ashley C. Banyard[3], Scott M. Reid[3], Andrew Brownlow[4], Mark Wessels[5]
[1]University of Liverpool, Liverpool, United Kingdom, [2]Cornwall Marine Pathology Team, Falmouth, United Kingdom, [3]Animal and Plant Health Agency, Weybridge, United Kingdom, [4]University of Glasgow, Glasgow, United Kingdom, [5]Finn Pathologists, Weybread, United Kingdom

Naturally occurring Influenza A virus (IAV) infection of both grey and harbor seals involving different strains of low pathogenicity IAV is associated with respiratory tract infection, and sporadic increases in mortality. Since 2020, extensive wild bird mortality events in sea birds due to high pathogenicity avian influenza (HPAI) H5Nx, has resulted in increased infection pressure on different seal and marine mammal species globally, often resulting in high mortality characterized by encephalitis, with or without visceral tissue involvement.

Necropsy of seals from the UK coast revealed HPAI positive (RT-PCR) brain tissue in seven animals (grey and harbor seals). Histological examination in all but one revealed variable patterns of meningitis and/or encephalitis, similar to previously reported cases of H5Nx infection in seals (lymphohistiocytic meningitis, perivascular cuffing, neuronal necrosis, gliosis); one seal also exhibited choroiditis and ventriculitis. Immunohistology for IAV antigen revealed variable viral antigen distribution and in addition to neurons and glial cells, virus antigen was detected in meninges, perivascular inflammatory cells, endothelial cells, choroid and ependyma cells.

IAV encephalitis in other mammalian species is associated with infection of the olfactory epithelium and anterograde spread of virus to the olfactory bulb. Due to the caudal distribution of the olfactory epithelium within the nasal cavity, nasal swabs for detection of IAV RNA may not be diagnostic, which has both epidemiological and personnel safety implications for HPAI detection in pinnipeds. Furthermore, unless examination of brain tissue is carried out (either by RT-PCR, or immunohistology), cases of IAV in pinnipeds may be under reported.

68: RIGHT ATRIAL RUPTURE SECONDARY TO CARDIAC HEMANGIOSARCOMA IN A DOG

Laura Machado Ribas, Kiersten Forsyth, Andrea Pires dos Santos, Luis Dos Santos
Purdue University, West Lafayette, IN, USA

Background: Hemangiosarcoma, dogs' most common cardiac neoplasia, primarily affects the right atrium (RA) and auricle. RA rupture is a rare condition in humans reported secondary to trauma or neoplasia, particularly angiosarcoma. In veterinary patients, RA rupture has only been reported secondary to trauma.

CASE PRESENTATION: A 14-year-old female spayed Australian Shepherd dog presented emergently after an episode of collapse, possibly stroke. On presentation, tachycardia, muffled heart sounds, and abnormal mentation were noted. Point-of-care ultrasound revealed pericardial effusion; 49 mL of fluid was drawn. Fluid analysis was consistent with hemorrhagic effusion, although cardiac puncture could not be excluded. Complete blood count showed mild anemia, lymphopenia, and neutrophilia. Thoracic radiographs pointed to alveolar pattern in the left cranial lung field and mild pleural effusion, suspected to be due to a mediastinal mass. Transthoracic echocardiography unveiled moderate pericardial effusion with cardiac tamponade and a large structure, suspected to be a mass, originated from the RA, with evidence of clot formation and RA wall rupture with flow from within the atrium into the pericardial space — the owners elected for humane euthanasia and necropsy. Gross pathology revealed four small to large tears within the RA rimmed by friable tissue suspected of hemangiosarcoma. Histopathology and immunohistochemistry (CD31) confirmed the presence of hemangiosarcoma within the lungs, RA, and left ventricle. This is the first case of atrial rupture secondary to neoplasia in dogs, which unfortunately holds a poorer prognosis as surgery is not advised due to the friability of the tissue and high likelihood of metastasis.

69: UNDERSTANDING THE ROLE OF ONCOGENIC MYC SIGNALING IN THE CANINE METASTATIC OSTEOSARCOMA TUMOR IMMUNE MICROENVIRONMENT

Rebecca Makii, Daniel Regan
Flint Animal Cancer Center, Fort Collins, CO, USA

Background: Osteosarcoma (OS) is the most common primary malignant bone tumor in both humans and dogs. Despite aggressive treatment, up to 30-40% of children and >90% of dogs die due to multi-drug resistant metastatic disease. MYC is a nuclear transcription factor that regulates a variety of cellular functions, and its amplification is associated with poor outcome in human OS. Recent data in mouse models of lung cancer has demonstrated MYC overexpression modulates the tumor immune microenvironment by increasing tumor-associated macrophage (TAM) influx and driving T lymphocyte exclusion.

Objective: We hypothesize MYC overexpression in metastatic canine OS contributes to an immunosuppressive environment by driving expression of chemokines that promote TAM enrichment and inhibit T cell recruitment.

Method: 42 archived FFPE lung metastatic canine OS samples were evaluated for MYC copy number variation (CNV), mRNA, and protein expression via ddPCR, Nanostring gene expression analysis, and immunohistochemistry (IHC) for MYC. 7 samples also underwent GeoMX spatial profiling to more specifically evaluate T cell and macrophage transcriptional profiles.

Results: We demonstrate that quantitative protein analysis via IHC, rather than gene copy number, is a better indicator of MYC hyperactivity in canine metastatic OS. Patient stratification based on MYC protein expression demonstrates that 'MYC-high' tumors are associated with downregulation of cytotoxic effector T-cell associated transcripts and upregulation of TAM transcripts.

Conclusion: This data suggests while MYC overactivity in metastatic canine OS may not be genomically driven, other mechanisms that lead to increased MYC protein expression are associated with transcriptomic profiles supportive of local immunosuppression.

70: NOVEL DEMONSTRATION OF INTERFACE DERMATITIS AND CORYNEBACTERIUM BOVIS-ASSOCIATED LESIONS IN NATURALLY INFECTED ATHYMIC NUDE RATS (RATTUS NORVEGICUS)

Renata Mammone[1], Pedro Ruivo[2], Neil Lipman[1], Ileana Miranda[1]
[1]Laboratory of Comparative Pathology, Memorial Sloan Kettering Center, Weill Cornell Medicine, and The Rockefeller University, New York, NY, USA, [2]Comparative Pathology Laboratory, University of California, Davis School of Veterinary Medicine, Davis, CA, USA

Background: *Corynebacterium bovis* (Cb) is a Gram positive, lipophilic short rod that commonly causes skin lesions in immunodeficient mice and was recently associated with similar lesions and mortality in Armenian hamster neonates. The typical clinical presentation of Cb infection is a flaking dermatitis characterized histologically by orthokeratosis, acanthosis, intracorneal bacterial colonies, and variable dermal inflammation. Though previous studies demonstrated Cb colonization in rats, there is no peer-reviewed literature describing clinical disease or histopathologic lesions resulting from Cb infection in this species.

Objective: This retrospective study aims to describe the histologic features of rats with skin disease submitted to the Laboratory of Comparative Pathology from Jan 2018 to Dec 2023 and correlate these findings with culture results.

Methods: Skin lesions were further evaluated through immunohistochemistry for inflammatory cell infiltrates and in-situ hybridization for Cb.

Results: Histopathology revealed variable degrees of orthokeratosis (53/53), acanthosis (53/53), interface dermatitis and/or folliculitis (47/53), and presence of bacteria visible on H&E and/or Gram staining (38/53). Other lesions considered secondary included furunculosis, epidermal pustules, dermal edema, and perivascular dermatitis. Features of interface dermatitis/folliculitis were primarily composed of CD3+ T lymphocytes and Iba1+ macrophages along the basement membrane of the epidermis and/or hair follicles. Cb was isolated from the skin in 42/46 rats, but ISH staining colocalized with lesions in only 3/19 rats.

Conclusion: While Cb may contribute to the development of interface dermatitis, the involvement of other bacteria or additional components of the skin microbiome cannot be ruled out.

71: HISTOLOGICAL FINDINGS IN THE CARDIOVASCULAR SYSTEM OF AGING AFRICAN GREEN MONKEYS (CHLOROCEBUS AETHIOPS SABAEUS) IN ST. KITTS

Regina Kemper[1], Andrew Curtis[1], Geshini Perera[1], Pompei Bolfa[1,2], Diana Bochynska[1,2], Matthew Valentine[1,2], Georgios Paraschou[1,2], Maria Jose Navarrete-Talloni[1,2]
[1]Ross University School Veterinary Medicine (RUSVM), Pathology Division, Biomedical Sciences Department., Basseterre, Saint Kitts and Nevis, [2]Ross University School Veterinary Medicine (RUSVM), Research Center for Integrative Mammalian Research., Basseterre, Saint Kitts and Nevis

BACKGROUND

Cardiovascular disease has long been recognized as an important and persistent cause of non-infectious morbidity and mortality in managed primate populations in biomedical research facilities. Few studies have investigated age-related conditions as the underlying cause of cardiovascular disease in primates, including the description of background lesions. The African green monkey

(AGM, *Chlorocebus aethiops sabaeus*) has served as an animal model for studying hypertension, however, current interest lies in understanding the background lesions and age-related histological findings in the cardiovascular system, as it plays an important role in the proper interpretation of studies using this species as an animal model.

MATERIALS & METHODS

Twenty-two AGM were euthanized and submitted to the RUSVM Pathology department. Standardized autopsies were conducted, and gross lesions were recorded. Tissue samples of the aorta (thoracic and abdominal) and heart, among other tissues, were collected and fixed in 10% buffered formalin. Samples were routinely processed, embedded in wax-paraffin, and stained with HE. Additional special histochemical stainings were requested if necessary.

RESULTS

Lesions spanned from thickening of the tunica media attributed to concentric hypertrophy/hyperplasia (suggestive of hypertension) and arteriolosclerosis in medium to small arteries, to cardiomegaly or cardiac hypertrophy, fibrosis, thrombosis, myocarditis, lipofuscin accumulation and myocardial degeneration in the heart.

CONCLUSION

The cardiovascular findings in this study highlight the importance of age-related or non-age-related lesions, that could be similar to those found in humans and other animal species. Being able to differentiate these will benefit pathologists who work routinely with AGMs in the research setting (pharmaceutical and biomedical).

72: ADENOCARCINOMA ORIGINATING FROM THE LACRIMAL GRAND WITH METASTASIS TO MULTIPLE ORGANS IN A RABBIT (ORYCTOLAGUS CUNICULUS)

Naka Okumura[1], Timothy Brunner[2], Javier G Nevarez[2], Bianca Santana de Cecco[3], Nanny Wenzlow[3]
[1]Nihon University, Kanagawa, Japan, [2]Louisiana State University, Baton Rouge, LA, USA, [3]Louisiana Animal Diagnostic Disease Laboratory, Baton Rouge, LA, USA

Background and Objective: Malignant tumors originating from the lacrimal glands are uncommon in domestic animals. Although they have been reported in some species and one case of metastasis in a cat in veterinary medicine, information on tumors originated from lacrimal glands in rabbits is scarce. This study describes the clinical and pathological characteristics of a metastatic lacrimal gland adenocarcinoma in a rabbit.

Signalment: An 11-year-old female spayed American rabbit (*Oryctolagus cuniculus*) weighing 2.1 kg, presented to the Louisiana State University Zoological Medicine service due to a left maxillary mass. Due to a poor prognosis, the rabbit was euthanized and submitted for a postmortem examination.

Results: Grossly, the subcutaneous tissue of the left maxilla was expanded by a nodule, which invaded into the left orbit. Similar nodules were noted in the lungs, kidneys, and liver. Histologically, all nodules were poorly demarcated, unencapsulated, highly infiltrative, and densely cellular consisting of neoplastic cuboidal to polygonal cells arranged in acini and occasional tubules anchored in a delicate fibrous stroma. Neoplastic cells were positive for anti-keratin/cytokeratin AE1/AE3 antibody. Considering the location and anatomopathological features this neoplasm was diagnosed as a lacrimal gland adenocarcinoma.

Conclusion: This study described the anatomopathological and immunohistochemical characteristics of a metastatic lacrimal gland adenocarcinoma in a rabbit. Given the anatomy of the lacrimal gland in

rabbits, lacrimal gland tumors should be considered not only when the tumor is intraorbital but also when it is extraorbital.

73: DECREASED PHOSPHORYLATED STAT5 MAY CONTRIBUTE TO MALIGNANCY IN FELINE MAMMARY CARCINOMA, AND INCREASED PHOSPHORYLATED STAT3 IS A POSSIBLE FACTOR OF ITS POOR PROGNOSIS

Keishi Owaki[1], Mami Murakami[2,3,4], Kana Kato[3], Kentaro Okamoto[3], Akihiro Hirata[1,3,4], Hiroki Sakai[1,3,4]
[1]Laboratory of Veterinary Pathology, Joint Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan, [2]Laboratory of Veterinary Clinical Pathology, Joint Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan, [3]Laboratory of Veterinary Pathology, Joint Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan, [4]Center for One Medicine Innovative Translational Research (COMIT), Gifu University, Gifu, Japan

Background & **Objective:** Feline mammary carcinoma (FMC) is an aggressive metastatic cancer with a poor prognosis. FMC shares many characteristics with human breast cancer, making it a valuable model for studying human breast cancer biology. Unlike human breast cancer, the mechanisms underlying FMC development are poorly understood, which limits the development of effective therapies. The abnormal activities of signal transducers and activators of transcription (STAT) 3 and STAT5 has been reported in human breast cancer. This study aimed to examine the role of STAT3 and STAT5 in FMC development.

Methods: Tissues from 80 cats with mammary carcinoma were subjected to immunohistochemical staining with antibodies against total STAT3 (tSTAT3) and STAT5 (tSTAT5), phosphorylated STAT3 (pSTAT3), and STAT5 (pSTAT5). The labeling index (LI) of pSTAT3 and pSTAT5 in normal mammary glands, mammary hyperplasia, adenoma, and carcinoma were assessed. The relationship between pSTAT3 LI and clinicopathological data was analyzed using the Steel–Dwass tests and log-rank tests.

Results: tSTAT3 and tSTAT5 were detected in neoplastic and non-neoplastic mammary tissues. pSTAT5 LI in FMC tissues was significantly lower than that in adenoma and non-neoplastic mammary tissues; the pSTAT3 LI in FMC tissues was significantly higher. Furthermore, STAT3 activation was associated with metastasis and tumor-related survival time were significantly shortened in cats with FMCs with higher pSTAT3 LI.

Conclusion: Decreased STAT5 activation may contribute to FMC development. Conversely, increased STAT3 activation is linked to tumorigenesis and metastasis, indicating a potential correlation with shorter survival in cats with FMC.

74: CANINE MORBILLIVIRUS INFECTION OF PRIMARY ALVEOLAR MACROPHAGES PROVIDES INSIGHTS INTO MECHANISMS OF VIRAL INTERFERENCE WITH INNATE IMMUNE DEFENSE RESPONSES

Pauline Poepperl[1], Elisa Chludzinski[1], Melanie Stoff[1], Hannah Gerhards[1], Martin Ludlow[1], Robert Geffers[2], Andreas Beineke[1]
[1]University of Veterinary Medicine Hannover, Foundation, Hannover, Germany, [2]Helmholtz Centre for Infection Research, Braunschweig, Germany

Background

Canine morbillivirus (formerly: canine distemper virus, CDV), a highly contagious morbillivirus with a broad host range, causes severe immunosuppression in naturally infected animals, favoring the

development of secondary bacterial and viral infections. The anatomic localization of alveolar macrophages (AMs) in the lungs means that these cells are speculated to have a significant role as initial target cells and in lung lesion development.

Objectives

This study aimed to characterize viral replication and cytopathic effects in response to infection with different CDV strains and to study cellular responses, emphasizing the role of viral interference with host innate immune signaling.

Methods

Primary canine AMs were infected with CDV, and cellular morphology and virus infection rates were analyzed. Molecular methods and transcriptome analysis were used to characterize both the cellular response and degree of virus replication.

Results

Infection rates were higher in AMs infected with the CDV R252 strain, while cytopathic effect was more pronounced in CDV Onderstepoort (Ond)-infected AMs. CDV Ond-infected cells produced stronger pro-inflammatory responses with increased TNF-α expression. Moreover, RNA sequencing revealed a dominance of type I interferon responses caused by CDV infection, preferentially in Ond-infected cells.

Conclusion

Primary canine AMs can be efficiently and productively infected by CDV, leading to pro-inflammatory responses. Accelerated viral elimination following infection with the attenuated CDV Ond strain is related to increased antiviral type I interferon responses. Differential gene expression of AMs infected with different strains reveals insights in mechanisms of viral interference with innate immune signaling and viral attenuation.

75: MOLECULAR CHARACTERIZATION OF SARCOCYSTIS NEURONA IN CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) WITH POLYPHASIC RHABDOMYOSITIS

Alene Pohly[1], Devinn Sinnott[1], Margaret Martinez[2], Cara Field[2], Pádraig Duignan[2], Karen Shapiro[1]
[1]University of California Davis, Davis, CA, USA, [2]The Marine Mammal Center, Sausalito, CA, USA

A unique clinical syndrome characterized by polyphasic rhabdomyositis associated with *Sarcocystis neurona* has been reported in both free-ranging and captive California sea lions (CSLs; *Zalophus californianus*). The pathogenesis of this disease is poorly understood. Specific *S. neurona* genotypes have been associated with more severe sarcocystosis and mortality in other marine mammals. *Sarcocystis neurona* genotypes in CSLs with rhabdomyositis have not yet been investigated and the role of parasite genotype as a driver of this disease is unknown. The objective of this study was to characterize the genotypes of *S. neurona* present in CSLs with rhabdomyositis and to compare the genotypes present in CSLs to *S. neurona* genotypes present in other marine and terrestrial hosts. Archived skeletal muscles of affected CSLs were screened for *S. neurona* DNA using polymerase chain reaction (PCR) targeting a sensitive, multi-copy locus (ITS1), followed by genotyping using PCR-based multilocus sequence typing at six loci. Genotypes identified in CSLs were compared to previously published genotypes reported from sea otters, opossums, and horses. In 12 CSLs, nine different genotypes of *S. neurona* were identified. Of these, four genotypes have been previously identified in southern sea otters (*Enhydra lutris nereis*). The remaining five genotypes have not been previously identified in any host to date. This study demonstrates that diverse genotypes of *S.*

neurona are present in free-ranging CSLs affected with rhabdomyositis, and that CSLs share some overlapping *S. neurona* genotypes with other hosts, but also appear to be infected with unique genotypes that have not been previously reported.

76: TESTICULAR TUMORS IN REPTILES: A RETROSPECTIVE STUDY OF CASES SUBMITTED TO TWO SPECIALTY DIAGNOSTIC SERVICES

Alisha Pushinsky[1], Michael Garner[2], Robert Ossiboff[1]
[1]Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, [2]Northwest ZooPath, Monroe, WA, USA

There is a paucity of literature on testicular tumors in non-avian reptiles, with most documentations restricted to single case studies or grouped within general retrospective studies of reptile neoplasms. As populations of reptiles continue to decline, managed reptiles in private or zoological collections will likely play an important part in better understanding states of reproductive development and disease. To better understand the types of reproductive neoplasia in male reptiles, a retrospective study of testicular neoplasia was performed utilizing cases received from two specialty diagnostic services (Northwest ZooPath [1998 – 2022] and the Aquatic, Amphibian, and Reptile Pathology Service at the University of Florida [2017 - 2024]). A total of 64 testicular tumors within 62 cases were identified. Testicular tumors were categorized as either primary (seminomas, Leydig cell tumors, Sertoli cell tumors, mixed germ cell tumors), unclassified, adnexal, and/or epididymal testicular tumors. Seminomas were the most common diagnosed tumor type, representing 41.4% (12/29) of all testicular tumors in snakes, 50% (14/28) in lizards, all (3/3) testicular tumors in chelonians, and in 25% (1/4) in crocodilians. Other common lesions that can grossly appear like neoplasms, including cysts, ovotestes, testicular hyperplasia, and xanthomas, were also assessed and identified in 17 cases. To the authors' knowledge, this retrospective study is the first to focus specifically on neoplasia and neoplasia-like lesions in the reptile testicle, and will offer important baseline information on male reptile reproductive disease in captivity.

77: DETECTING EAR LESIONS IN SLAUGHTERED PIGS USING ARTIFICIAL INTELLIGENCE

Matteo D'Angelo[1], Domenico Sciota[1], Alfonso Rosamilia[2], Chiara Guarneri[3], Chiara Cecchini[1], Jasmine Hattab[1], Marino Marà[1], Anastasia Romano[1], Alberto Olivastri[4], Giuseppe Marruchella[1]
[1]University of Teramo, Teramo, Italy, [2]Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna, Brescia, Italy, [3]Azienda Unità Sanitaria Locale, Modena, Italy, [4]Azienda Sanitaria Territoriale, Ascoli Piceno, Italy

Background: Recording skin lesions in slaughtered pigs is useful to assess animal welfare.

Objectives: Training a convolutional neural network (CNN) to detect ear lesions at slaughter.

Methods: A total of 1415 pictures (dataset) were randomly collected along the slaughter chain. Carcasses were photographed after passing through the scalding tank, flaming and brushing. Each image included the external surface of both ears and was evaluated by two veterinarians, who agreed to classify pinnae as healthy or diseased. Moreover, ears ripped after brushing were classified as unsuitable. Thereafter, 1000 images were used to train an open-source CNN (TensorFlow, available at https://keras.io) to detect any change of the auricle silhouette. The remaining 415 images were used to test CNN's accuracy, when comparing its predictions with veterinarians' assessment.

Results: Veterinarians classified 78.2% ear pinnae as healthy, 17.8% as diseased and 4.0% as unsuitable. Overall, CNN's accuracy was 95,06%. However, CNN was much less accurate to correctly identify unsuitable ears (59,45%). More in detail, CNN correctly recognized ear pinnae affected by severe artifacts (22 out of 37), while it mistakenly identified as healthy (n = 7) or diseased (n = 8) the remaining 15 unsuitable ears.

Conclusions: This study highlights that open-source, artificial intelligence-based tools could effectively fulfill well-targeted tasks. Artifacts represented the greatest concern, as they were the main responsible of mistakes. We consider such an issue could be solved by increasing the dataset and/or taking pictures before brushing, whether allowed by the slaughter chain facility.

78: ENTAMOEBIASIS IN A CAPTIVE POPULATION OF ALLIGATOR NEWTS (ECHINOTRITON ANDERSONI)

Varvara Semenova[1], Nicholas Buscaglia[2], Robert Ossiboff[1]
[1]University of Florida College of Veterinary Medicine, Gainesville, FL, USA, [2]Sedgwick County Zoo, Wichita, KS, USA

Entamoeba spp. are known to cause severe enteric and sometimes systemic disease in a multitude of domestic and nondomestic species. In amphibians, *Entamoeba* spp. are generally documented as incidental gastrointestinal parasites, with rare reports of clinically significant Entamoebiasis. In the last five years, mortality outbreaks caused by a recently described protozoa, *Entamoeba* sp. CT1, have been documented in managed anurans in the USA and in free-ranging anurans in Australia. In both reports, severe necroulcerative to proliferative gastroenteritis resulted in substantial morbidity and mortality. In 2023, a managed, zoological population of endangered alligator newts (*Echinotriton andersoni*) experienced a similar outbreak, with the death or euthanasia of 5/13 (38%) newts over a period of nearly three months. Noted clinical signs included dehydration, poor skin quality, and lethargy. Microscopic examination of the first three newts revealed marked, proliferative and necrotizing inflammation of the intestinal tract and cloaca with intramucosal amoebic protozoa. The gonadal ducts (2/3) and urinary bladder (2/3) were also affected in a subset of animals. PCR and Sanger Sequencing of formalin-fixed paraffin embedded intestinal tissue from one newt amplified DNA with 100% sequence match to *Entamoeba* sp. CT1. In contrast to the recent reports of *Entamoeba* sp. CT1 in anurans, the lesions in these caudates were not restricted to the gastrointestinal tract. This is the first report of *Entamoeba* sp. CT1 causing significant clinical disease in caudates, emphasizing it as an important amphibian pathogen with the potential to cause disease in managed and free-ranging amphibian populations globally.

79: BRONCHOALVEOLAR LAVAGE FLUID CYTOKINE PROFILES IN SEVERE EQUINE ASTHMA

Emma Stacey[1], Silvana Beljo[1], William Ibbott[1], Heng Kang[1], William Sears[2], Janet Beeler-Marfisi[1]
[1]Department of Pathobiology, Ontario Veterinary College, Guelph, ON, Canada, [2]Department of Population Medicine, Ontario Veterinary College, Guelph, ON, Canada

Severe equine asthma (SEA) is a common, non-infectious, inflammatory disease of the distal airways in horses with an estimated prevalence of 20% in the Northern Hemisphere. Exposure to organic and inorganic particles found in moldy hay, such as fungi, molds, and endotoxin, are known to induce asthma in susceptible horses.

The goal of this study was to examine cytokine profiles in bronchoalveolar lavage fluid (BALF) in asthmatic and non-asthmatic horses. Seven horses with SEA and 7 control horses were sampled at time zero and after 48-hours of exposure to moldy hay. Multiplex analysis of 23 cytokines and chemokines, FGF23, eotaxin, G-CSF, IL-1α, GM-CSF, fractalkine, IL-13, IL-5, IL-18, IL-1β, IL-6, IL-17A, IL-2, IL-4, IL-12p70, IFN-γ, IL-8, IP10, GRO (CXCL1), MCP1 (CCL2), IL-10, TNF-α, and RANTES, was performed on BALF collected at both time points.

Fractalkine, IFN-γ, IL-8, IL-18, IL-5, GRO, MCP1, and TNF-α were consistently detected among horses and statistical analysis comparing the concentrations between control and asthmatic groups

before and after exposure was performed. Only GRO (CXCL1) and MCP1 (CCL2) showed a significant difference between asthmatic and non-asthmatic horses, with increased concentrations detected in SEA horses ($p < 0.05$). Increases in these chemokines makes biological sense because they facilitate the influx of neutrophils and monocytes to sites of inflammation, respectively, and these leukocytes characterize the cytologic profile of SEA. Preliminary analysis of RNA-seq data from alveolar macrophages suggests genes for similar cytokines are also upregulated. Future work will study why asthmatic horses have these increases while healthy horses do not.

80: MORBIDITY AND MORTALITY OF FREE-RANGING AMERICAN BLACK BEARS (URSUS AMERICANUS) IN THE EASTERN UNITED STATES FROM 1975-2023

Robert Stilz[1,2], Melanie Kunkel[3], Kevin Keel[4], Heather Fenton[5], Alisia Weyna[6], Kevin Niedringhaus[7], Michael Yabsley[1], Mark Ruder[1], Nicole Nemeth[1,2]
[1]Southeastern Cooperative Wildlife Disease Study, Athens, GA, USA, [2]University of Georgia College of Veterinary Medicine, Athens, GA, USA, [3]Northeast Association of Fish and Wildlife Agencies, Ithaca, NY, USA, [4]University of California Davis School of Veterinary Medicine, Davis, CA, USA, [5]Taronga Conservation Society, Mosman, Australia, [6]University of Wisconsin School of Veterinary Medicine, Madison, WI, USA, [7]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA

Background: American black bears are a keystone species in North America, playing a critical role in seed dispersal and wild herbivore population control. They are also of high public interest and are an important game species.

Objective: To characterize morbidity and mortality causes among wild black bears submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS).

Methods: Gross, histologic, and laboratory findings were reviewed from 243 black bear cases from 12 states submitted to SCWDS from 1975-2023.

Results: 50.2% (n=122) were male; 43.6% (n=106) were female, and sex was unknown for 6.2% (n=11). 53.9% (n=131) were adults (3+ years); 4.5% (n=11) were subadults (2-3 years); 16% (n=39) were yearlings (1 year); 18.5% (n=45) were cubs (<1 year), and age was unknown for 7% (n=17). The most common diagnoses included trauma (26.7%, n=65), sarcoptic mange (25.1%, n=61), and emaciation (20.6%, n=50). The most common causes of trauma were vehicle collision (10.7%, n=26) and gunshot wound (7.8%, n=19). Less common diagnoses included infectious disease (10.3%, n=25), idiopathic inflammatory disease (9.5%, n=23), neoplasia (4.5%, n=11), and toxicosis (1.6%, n=4). In 2023, one bear was diagnosed with H5N1 highly pathogenic avian influenza virus. 24 (9.9%) and 14 bears (5.8%) were dispatched for nuisance or aggressive behaviors, respectively.

Conclusions: Morbidity and mortality in wild black bears are often associated with human proximity, whether through overt anthropogenic trauma or environmental changes that may deplete suitable food resources, leading to emaciation, secondary infectious diseases, and promotion of perceived nuisance or aggressive behaviors.

81: A SURVEY OF TRYPANOSOMA CRUZI IN THE BLACK-EARED OPOSSUM (DIDELPHYS MARSUPIALIS), RED-RUMPED AGOUTI (DASYPROCTA LEPORINA) AND NINE-BANDED ARMADILLO (DASYPUS NOVEMCINCTUS) IN TRINIDAD, WEST INDIES

Rod Suepaul[1], Karelma Acevedo[1], Azad Mohammed[2], Jennifer Peterson[3], Nicole Gottdenker[4], Indira Pargass[1], Devina Supersad[1], Krista Ali[1], Vrijesh Tripathi[2], Adesh Ramsubhag[2], Christopher Oura[1]
[1]The University of the West Indies, School of Veterinary Medicine, Champs Fleurs, Trinidad and Tobago, [2]The University of the West Indies, Department of Life Sciences, St. Augustine, Trinidad and Tobago, [3]College of Agriculture and Natural Resources, University of Delaware, Newark, DE, USA, [4]University of Georgia, College of Veterinary Medicine, Athens, GA, USA

Background: *Trypanosoma cruzi* (Chagas disease) circulates in Trinidad but has been documented only in its arthropod vector and in one rat. Opossum, armadillo, and agouti are present in Trinidad and are known *T. cruzi* hosts.

Objective: To demonstrate *T. cruzi* infection in opossum, armadillo, and agouti in Trinidad.

Methods: Tissues from 75 opossums, 20 armadillos, and 23 agouties collected throughout Trinidad between 2014 and 2020 were analyzed for *T. cruzi* infection. Diagnostic methods included PCR on the heart and spleen (all species); blood smear examination (opossum and armadillo); immunofluorescence (opossums); and histopathology (opossum and armadillo).

Results: For opossum, 81% (51/63) of splenic samples and 79% (42/53) of heart samples were *T. cruzi*-positive via PCR; immunofluorescence revealed a 39% (22/57) seropositivity; all blood smears were negative (0/72); lymphohistiocytic myocarditis was found in 15% (9/61) opossums examined. Overall, 32% of opossums (24/75) were positive for *T. cruzi* via two different diagnostic methods. For armadillo, 37% (7/19) of splenic samples and 53% (10/19) of heart samples were *T. cruzi*-positive via PCR; trypanosomes were observed in the blood of one animal (1/15); lymphohistiocytic myocarditis was observed in 25% (4/16) of armadillos. Overall, 20% of armadillos (5/20) were positive for *T. cruzi* via two different diagnostic methods. In agouti, 83% (15/18) of splenic samples and 75% (12/16) of heart samples were PCR positive for *T. cruzi*.

Conclusion: This is the first report of *Trypanosoma cruzi* in wild reservoirs in Trinidad. The hunting and consumption of these animals poses a possible risk of Chagas disease transmission.

82: OUTBREAK OF HSV-1 IN FREE-RANGING NON-HUMAN PRIMATES IN CENTRAL-WEST BRAZIL

Regina Kemper[1], João Cavasani[2], Lucas Pavelegini[2], Luciano Nakazato[2], Valeria Dutra[2], Marcio de Castro[3], Edson Moleta Colodel[2], Fernando Furlan[2]
[1]Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis, [2]Federal University of Mato Grosso, Cuiaba, Brazil, [3]University of Brasilia, Brasilia, Brazil

Background:

Herpes Simplex Virus (HSV) is a primary human pathogen. Non-human primates (PNHs) infection occurs after contact with humans carrying the virus. The disease occurs frequently in captive PNHs and rarely in free-ranging PNHs. Thirteen free-ranging *Mico melanurus* and one *Aotus azarae* were referred to the Veterinary Hospital of the Federal University of Mato Grosso, Brazil. Three of them were still alive and presented eyelid ptosis, mydriasis, incoordination, ataxia, dysmetria, opisthotonos, and muscular tremors. The animals were from an urban forest fragment and had frequent contact with humans.

Objective:

To describe the pathological, molecular, and immunohistochemical findings in an outbreak of HSV-1 in PNHs.

Methods:

Postmortem examination was performed, and samples were collected and processed routinely for histologic evaluation. Polymerase Chain Reaction (PCR) HSV-1 and HSV-2 were conducted and sequenced. For the immunohistochemistry (IHC), a polyclonal rabbit antibody against HSV-1 and HSV-2 were used.

Results:

The autopsy findings were hyperemia of the leptomeninges and multifocal ulcerative glossitis. Microscopically, the primary lesions were non-suppurative meningoencephalitis, hepatitis, and glossitis with intranuclear inclusion bodies.

All the brain samples were positive in IHC and/or PCR, and the positivity was 86.4% and 77.8% of the cases, respectively. Phylogenetic analysis revealed a high identity with HSV-1 belonging to the same clade.

Conclusion/discussion

The investigation confirmed an HSV-1 outbreak in PNHs. Unlike observed in humans and Old-World primates, infection in New World primates results in a severe and usually fatal clinical condition with non-suppurative meningoencephalitis, hepatitis, and glossitis. Proximity with humans can lead to disease spillover.

83: THE ROLE OF MIRNAS IN THE REGULATION OF INNATE IMMUNITY IN CATTLE AND HORSES

Andrii Trokoz[1], Russell Fraser[2], Geoffrey Wood[1], Luis Arroyo[1], Brandon Lillie[1]
[1]University of Guelph, Guelph, ON, Canada, [2]University of Prince Edward Island, Charlottetown, PE, Canada

In the Canadian dairy, beef, and horse industries, infectious diseases represent a substantial economic and health threat, causing significant annual losses. To defend against pathogens, the host's innate immune system acts as the first line of defense, involving various mechanisms such as physical barriers, antimicrobial peptides, and pattern recognition receptors (PRRs). PRRs, including Toll-like receptors, NOD-like receptors, RIG-I-like receptors, and collagenous lectins rapidly detect pathogen-associated molecular patterns, triggering immune responses. MicroRNAs(miRNAs), via their impact on gene expression, influence cytokine production, adhesion molecule expression, and expression of PRRs. The liver is a central player in orchestrating and initiating innate immune responses by producing circulating PRRs, complement proteins, and other innate immune proteins. This study explored the role of miRNAs in regulating the innate immune system in cattle and horses. We hypothesized that miRNA expression varies in infected and noninfected animals, and miRNAs target genes involved in innate immune responses and that species-specific variability will be observed. The objectives include characterizing the differential hepatic expression of miRNAs with a focus on those that impact innate immune genes. RNA was extracted from liver tissue collected at postmortem from animals with and without infectious diseases. Isolated RNA underwent small RNA sequencing followed by bioinformatics analysis to investigate differential miRNA expression with prediction of novel miRNAs and identifying target genes for differentially expressed miRNAs. By unraveling the role of miRNAs in innate immune regulation, this study will contribute to our understanding of genetic regulation of innate resistance to infectious diseases in cattle and horses.

84: BONE FLUORIDE CONCENTRATION IN 2-YEAR-OLD DAIRY COWS WITH SPONTANEOUS HUMERAL FRACTURE FROM NEW ZEALAND

Alvaro S Wehrle-Martinez[1], Keren E Dittmer[1], Penny J Back[1,2], Chris Rogers[1,2], Jenny Weston[1], Paramsothy Jeyakumar[2], Richard Van Vleck Pereira[3], Robert Poppenga[4], Harry Taylor[5], Kevin E Lawrence[1]
[1]School of Veterinary Sciences, Massey University, Palmerston North, New Zealand, [2]School of Agriculture and Environmental Sciences, Massey University, Palmerston North, New Zealand, [3]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, [4]California Animal Health and Food Safety Laboratory System,

School of Veterinary Medicine, University of California-Davis, Davis, CA, USA, [5]Diagnostic and Surveillance Services, Biosecurity New Zealand, Ministry for Primary Industries, Upper Hutt, New Zealand

Background: Spontaneous fracture of the humerus caused by osteoporosis in primiparous dairy cows is a major issue in the dairy industry in New Zealand. It is hypothesized that the high concentration of fluoride in New Zealand's topsoil can affect the quality and strength of bones, contributing to the incidence of fractures.

Objective: Estimate the median fluoride content in bones from cows with humeral fracture (affected) and unaffected (control) cows and determine any likely association between bone fluoride concentration and spontaneous humeral fracture.

Methods: The fluoride concentration of humeral bone samples from 35 affected cows and 21 age-matched control cows was quantified using an Orion Fluoride ion selective electrode. A calibration curve determined the fluoride concentration in standards and samples based on mV response.

Results: Bones from affected cows had significantly higher median fluoride concentrations (490 mg/kg) than bone samples from control cows (290 mg/kg), with a p-value of 0.0006 based on the exact Wilcoxon rank-sum test.

Conclusions: Although cows affected by humeral fractures have higher median bone fluoride concentrations, the concentration is well below the levels associated with fluorosis. The increased fluoride concentration in bones from affected cows could be linked to the greater new bone formation observed in affected cows, and/or could have affected collagen synthesis, as decreased total collagen content has been reported in cows with spontaneous humeral fractures. Further investigation is essential to establish the potential impact of bone fluoride concentrations on the development of these fractures.

85: ADOMAVIRUS IS ASSOCIATED WITH MALIGNANT MELANOPHOROMA IN LARGEMOUTH BASS (MICROPTERUS SALMOIDES)

Mandy Womble[1], Clayton Raines[2], Heather Walsh[2], Gregory Lewbart[3], Luke Iwanowicz[4], Abigail Armwood[3]
[1]University of Illinois College of Veterinary Medicine Zoological Pathology Program, Brookfield, IL, USA, [2]U.S. Geological Survey, Kearneysville, WV, USA, [3]North Carolina State University College of Veterinary Medicine, Raleigh, NC, USA, [4]USDA Agricultural Research Service, Kearneysville, WV, USA

Largemouth bass (*Micropterus salmoides*) are apex predators in freshwater ecosystems in North America. Free-ranging and aquarium-housed largemouth bass can develop hyperpigmented melanistic skin lesions (HPMLs) that are associated with a novel adomavirus. Adomaviruses are related to papillomaviruses and polyomaviruses, viruses that are well known for their role in the development of hyperplastic lesions that can transition to malignant neoplasia in many species; however, malignant neoplastic transformation has not been described in association with adomaviruses. We report three cases of malignant melanophoroma in aquarium-housed largemouth bass with a possible association with adomavirus. Two bass from the same life support system (LSS) and another from a different location were afflicted with plaque-like pigmented ulcerative skin lesions that were diagnosed histologically as malignant melanophoromas with hepatic metastasis in two cases. Samples from two affected fish were screened for three different adomaviruses (*Micropterus salmoides* adomavirus [MsA]-1, MsA-2, and MsA-3) and were positive for MsA-1. Positive fluorescent RNAscope™ *in situ* hybridization signal was detected in dermal epithelial cells and in rare neoplastic melanocytes. In combination, these findings support an association between adomavirus infection,

HPMLs, and transformation to malignant neoplasia in largemouth bass. Future investigations into pathogenesis are needed to support a causative relationship; however, this is the first description of malignant neoplasia associated with adomavirus infection. The discovery of these putatively oncogenic viruses within both free-ranging and aquarium-housed largemouth bass has major implications for species management in aquaculture, natural ecosystems, and zoos, aquariums, and other institutions.

86: THE ROLE OF RAS SIGNALING PATHWAY IN TUMORIGENESIS OF FELINE INJECTION-SITE SARCOMA (FISS)

Yat Chong, Hui-Wen Chang, Yen-Chen Chang
Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan

Feline injection-site sarcoma (FISS) is an aggressive and invasive soft tissue sarcoma with an obscure pathogenesis but closely related to chronic inflammation. The aberrant activation of Ras signaling pathway, which operates downstream signaling pathways such as MAPK and P13K/Akt, is known to promote carcinogenesis in both human and veterinary cancers. This study aims to investigate the role of the Ras signaling pathway in FISS. The protein expression of pan-Ras, Erk 1/2, and Akt were evaluated in 34 clinical samples and 3 isolated primary FISS cells, and full-length sequencing of the Ras genes was also assessed. Our results demonstrated that pan-Ras, Erk, and Akt were overexpressed in varying degrees detected by immunohistochemistry stain and western blot when compared to normal feline muscle and skin tissue. There were several mutants observed, leading to three different amino acid substitution in H-Ras and K-Ras, but differed from the hotspots found in human cancer. Although the underlying mechanism remains unknown, we found that the Ras activation and cell function were altered in the FISS with mutants. In summary, the Ras signaling pathway may play an important role in the tumorigenesis of FISS and potentially provide novel insights for new therapeutic strategies or drug design.

87: IMMUNOCYTOCHEMICAL CHARACTERIZATION OF NEWLY CULTURED CELLS FROM 3 HIGH-GRADE OLIGODENDROGLIOMAS IN FRENCH BULLDOGS

Kio Yoshida, James Chambers, Kazuyuki Uchida
the University of Tokyo, Tokyo, Japan

Background: Canine oligodendroglioma has an oligodendroglial precursor cell (OPC)- and intermediate precursor cell (IPC)-like immunophenotype, suggesting that canine glioma has a pluripotent neural stem cell (NSC) lineage nature. To reveal NSC lineage characteristics *in vitro*, immunocytochemical analyses were performed on cultured cells from 3 biopsied high-grade oligodendrogliomas in French Bulldogs.

Methods: The biopsied tumor tissues were used for primary culture, and some were also fixed for histopathology. Tissue pieces were cultured under 2 different conditions as follows: DMEM/F12 and 10% FBS; DMEM/F12, 0.5 or 2% FBS, 20 ng/ml PDGF-AA, and 20 ng/ml FGF. Cultured cells were fixed with 4% PFA overnight at 4°C, and cell blocks of fixed cells were prepared. Cell blocks were routinely embedded in paraffin and subjected to immunocytochemistry.

Results: Based on the histopathologic features, all three cases were diagnosed as high-grade oligodendrogliomas. The tumor cells showed OLIG2 and PDGFRA immunolabeling, and a few cells showed CNPase immunolabeling. Immunocytochemistry of cultured cells revealed that a few cultured cells of passages 0-2nd showed OLIG2 immunolabeling. After several passages, OLIG2 immunolabeling of cultured cells disappeared completely. Cells cultured with PDGF-AA showed PDGFRA immunolabeling, whereas cells cultured with 10% FBS showed CNPase and β3-tubulin immunolabeling.

Conclusion: PDGF-AA may induce growth of OPC-like cells, whereas 10% FBS may induce differentiation of cultured cells into oligodendroglial and immature neuron-like cells. These findings suggest that canine oligodendroglioma has NSC lineage-like pluripotency. However, the loss of OLIG2 suggests that the culture conditions used are not sufficient to maintain the original natures of the tumors.