



1: SPLENIC EXTRASKELETAL OSTEOSARCOMA WITH HEPATIC METASTASIS AND SUBCUTANEOUS HEMANGIOSARCOMA WITH PULMONARY METASTASIS IN A DOG

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A 14-year-old, castrated male, mixed breed dog presented with a history of lethargy, inappetence, rapidly growing subcutaneous masses, and liver disease with icterus. Due to the deteriorating condition, humane euthanasia was elected. An autopsy released two subcutaneous masses along the axilla and sternum; one mass was dark red and the other mass was white-tan on cut sections.

There was hemoabdomen, and the head of the spleen was effaced by a firm, multinodular, mottled white-tan and dark red mass with partial rupture. Throughout all liver lobes were multifocal, firm, white-tan masses. Multiple lung lobes were infiltrated by soft, dark red masses. Histopathological, the splenic masses were composed of sheets of neoplastic spindle cells intermixed with numerous multinucleated giant cells. Multiple sections of the splenic neoplasm revealed rare foci of osteoid deposition. The hepatic neoplasms demonstrated similar features as those found in the spleen. The neoplastic spindle cells and multinucleated giant cells were negative for immunohistochemistry staining for CD31 and Iba-1. Histopathology of the subcutaneous masses revealed a subcutaneous hemangiosarcoma and a lipoma. The pulmonary neoplasms were hemangiosarcomas histopathologically. Given these findings and the lack of a primary bone neoplasm, a primary splenic extraskeletal osteosarcoma with hepatic metastasis and a subcutaneous hemangiosarcoma with pulmonary metastasis were diagnosed. Primary extraskeletal osteosarcoma arising from the spleen is rarely reported in dogs and should be considered as a differential diagnosis for splenic spindle cell neoplasms with multinucleated giant cells. Additional tissue sections for the identification of osteoid and immunohistochemistry can aid in the final diagnosis.

2: SYSTEMIC APOLIPOPROTEIN C-III AMYLOIDOSIS IN A WHITE LION FAMILY

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Background: Amyloidosis is a disease group caused by the amyloid formation of various precursor proteins. The precise identification of an amyloid precursor protein is necessary because the clinical approach differs for each amyloidosis type.

Apolipoprotein C-III (APOC3) amyloidosis has been reported only in humans, not in animals, and the pathogenesis involves a mutation in the *APOC3* gene.

Objective: We identified familial systemic amyloidosis in white lions (*Panthera Leo*) that had died in a Japanese safari park. This study aims to identify the amyloid precursor protein and characterize the pathologic features of amyloidosis in white lions.

Methods: Histopathologic evaluation was performed using H&E and Congo red staining.

LC-MS/MS and immunohistochemistry identified the amyloid precursor protein. RT-PCR and subsequent sequencing were performed to verify the mutations in the amyloid precursor protein.

Results: Of the five white lions available for analysis, four animals over 19 years of age had amyloid deposition in the kidneys or multiple organs; one 6-month-old animal was negative. Amyloid deposits were observed systemically and were characterized by severe at the renal corticomedullary junction. LC-MS/MS detected APOC3 as a dominant component of amyloid deposits, which were also positive for APOC3 by immunohistochemistry. Gene analysis revealed no mutations in the *APOC3* gene in amyloidosis-affected white lions.

Conclusions: We identified APOC3 amyloidosis for the first time in animals. The hallmark of this amyloidosis in white lions is a predominant deposition in the renal corticomedullary junction. Mutations in the *APOC3* gene do not appear to be associated with etiology in white lions.

3: SPENT OCULAR BLASTOMYCOSIS IN DOGS

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Background: Ocular blastomycosis in dogs is caused by *Blastomyces dermatitidis*; a dimorphic fungus that can impact the body systemically. Classically, the post-treatment eye has been considered a nidus for systemic re-infection, but this is not supported by published data. Histologic characterization of post-treatment “spent” ocular blastomycosis will provide a first step in studying whether the eye could be a nidus.

Objective: To histologically characterize spent ocular blastomycosis in domestic canines.

Methods: The histologic features reported for recent canine ocular blastomycosis COPLOW cases (2018-2023) in enucleated globes were reviewed. Inclusion criteria were clinical diagnosis of blastomycosis and description of treatment. Cases without yeast were re-examined with PAS for fungus stain.

Results: Twenty cases (24 eyes) met inclusion criteria. Yeast were identified in 17/24 eyes. Of cases with yeast, organisms were described as viable in 3 eyes, non-viable in 13 eyes, and both non-viable and viable in 1 eye. At the time of enucleation, 8/24 eyes had lymphoplasmacytic inflammation, 2/24 were suppurative, 4/24 were pyogranulomatous and 4/24 eyes were pyogranulomatous and lymphoplasmacytic, and 3/24 were granulomatous and lymphoplasmacytic. There was epichoroidal fibrosis in 13/24 eyes. Fibrovascular intraocular membranes were identified in 15/24 eyes, and 14/15 fibrovascular membranes were in the anterior segment. There were potentially blinding complications, including glaucoma diagnosed in 18/24 eyes and retinal detachment in 21/24 eyes.

Conclusions: Common features of spent ocular blastomycosis include non-viable yeast, lymphoplasmacytic and/or pyogranulomatous inflammation, epichoroidal fibrosis / anterior segment fibrovascular membranes, and retinal detachment.

4: IMMUNOHISTOCHEMISTRY OF SOFT TISSUE SARCOMA IN SNAKE SPECIES

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Background: Soft tissue sarcoma (STS) is a common neoplastic diagnosis in snakes but full histologic descriptions with immunohistochemistry are sparse.

Objective: To describe the tissue distribution, histologic patterns and identify the most specific and reliable immunohistochemical stains for differentiating snake STS.

Methods: Records from 37 histologically confirmed cases of soft tissue neoplasia in snakes submitted to the North Carolina State University Diagnostic Lab were reviewed.

Tissues were collected either for biopsy (13) or as part of a standard necropsy (24).

Results: Of the reported snakes included in this study, a majority (74%) were colubrids, followed by viperids (23%), and boids (3%). The average known age was 14 years (6-24 years). Biopsy submissions were either dermis or subcutis. For necropsies, the most commonly affected tissue were: heart (46%), subcutis (38%), and kidney (30%). Specific diagnoses were provided for 26 of the cases; the most frequent diagnoses were fibrosarcoma (5 necropsy and 8 biopsy) and leiomyosarcoma (5 necropsy). Smooth muscle actin, desmin, CD31, Factor VIII, myoglobin, and neuron-specific enolase were validated as specific and reliable while vimentin was nondiagnostic.

Conclusions: Snake STS is a common biopsy and necropsy diagnosis, and standard immunohistochemistry with the exception of vimentin can be used for further characterization. Leiomyosarcomas and fibrosarcomas are the most common diagnoses and skin / subcutis, heart and kidney the most commonly affected tissues.

5: CHARACTERIZING IMMUNOSUPPRESSION IN CANINE SARCOMAS

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Background: The checkpoint protein B7-H3 is expressed in human sarcoma cells and is associated with progression and metastasis. B7-H3 can also be induced on immune cells to promote immunosuppression. Peripheral immune cell B7-H3 expression has not been described in canine sarcomas and will aid design of novel B7-H3 targeted therapies.

Objective: To characterize B7-H3 expression in peripheral immunosuppressive cells and tumors from dogs diagnosed with sarcomas.

Methods: Peripheral blood mononuclear cells (PBMCs) were collected at diagnosis in dogs with primary soft tissue or bone sarcomas. Myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and B7-H3-expressing cells were quantified by flow cytometry. In a subset of dogs administered radiation therapy (RT), PBMCs were

serially analyzed after treatment. Spearman's correlation test, one-way ANOVA, or t-test were used to analyze cell subsets. B7-H3 expression in sarcoma tissue was assessed by immunohistochemistry in a pilot group of dogs undergoing RT.

Results/Conclusion: B7-H3 expression was detected on PBMCs in dogs with OSA (N=4), STS (N=5) and HSA (N=1). B7-H3+ monocytes and B7-H3+ T cells were detected in 8 and 6 dogs, respectively. There was no correlation between B7-H3+ cells and MDSCs or Tregs. Dogs with OSA had increased B7-H3+ cell subsets, polymorphonuclear (PMN) MDSC and Tregs compared to dogs with STS. In dogs (N=3) serially assessed after RT, B7-H3+ cell subsets and PMN-MDSC increased after RT. B7-H3 expression was strongly positive before and after RT in tumor tissue (N=4). Additional study is warranted to define B7-H3 expression and its contribution to immunosuppression.

6: DEVELOPMENT OF A HEART-ADAPTED TRANSFER LEARNING AI ALGORITHM FOR LESION DETECTION IN RAT SKELETAL MUSCLE

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Background

Deep learning artificial intelligence (AI) decision support tools for histologic assessment of toxicologic studies are typically developed in an organ-specific manner. Transfer learning (TL) aims to accelerate the development of a new model by utilizing an algorithm that was previously trained for a related task. For organs with similar histologic features, cross-organ TL may reduce the amount of input data needed to train a convolutional neural network to accurately identify normal and abnormal features of a new organ.

Objective

To develop a TL rat skeletal muscle classifier from a previously validated rat heart model and compare its performance to a novel classifier trained only on skeletal muscle.

Methods

Using Patholytix AI, two normal and three abnormal skeletal muscle tissue classes were annotated on 22 digitized slides across five studies. A novel classifier was trained on these annotations alone, and a TL model was trained on the same skeletal muscle annotations in addition to heart annotations previously used to develop a heart classifier. The classifiers were qualitatively evaluated on studies used for training and untrained studies.

Results

Overall, with equal effort, the heart-adapted TL skeletal muscle classifier identified lesions with greater sensitivity and specificity than the novel skeletal muscle classifier. The novel classifier identified many false positive areas of inflammation/infiltrate and necrosis/degeneration, missed some true necrotic lesions, and displayed some confusion between normal skeletal muscle and interstitium classes.

Conclusion

TL can accelerate the development of a new organ classifier from an algorithm previously validated in a histologically similar tissue.

7: TUMOR CALCINOSIS WITH IRON MINERALIZATION IN AN AFRICAN GREEN MONKEY

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A 19-year-old female African green monkey (*Chlorocebus sabaeus*) from a research colony was euthanized and submitted for autopsy after recurrent swelling on the right thigh, around the stifle, that started 5 years ago with unknown origin, resulting in lameness. The lesion was covered by alopecia and crusting and felt lobulated on palpation. Differential diagnoses included inflammation (granuloma) or neoplasia. Radiographic evaluation revealed multiple well-circumscribed, opaque mineralized foci within soft tissues extending from the right lateral hip to around the stifle, with no evidence of bone involvement, suggestive of soft-tissue calcification and/or ossification. Autopsy revealed a 11x5x4-cm, yellow-to-red, friable, crepitant, non-encapsulated multilobulated mass, infiltrating and replacing skeletal muscles of right thigh and containing multiple white hard spicules (mineralization).

Histologically, local skeletal muscles were replaced by abundant irregular-to-globoid amphophilic material containing combinations of calcium and iron (von Kossa and Perls' Prussian Blue positive) surrounded by extensive fibrous connective tissue (Masson's trichrome positive), admixed with numerous macrophages and fewer multinucleated giant cells (foreign body type). Several blood vessels were tortuous with thickened tunica media.

Our findings were most consistent with a diagnosis of tumoral calcinosis (TC), which has an unknown etiology in domestic animals, often with periarticular location, and may be inherited in people. Iron mineralization in chronic inflammation is a rare event and might be related to an Iron overload profile in some primates in the colony. TC with ectopic mineralization (calcium and Iron) should be included in the differential diagnosis of a radiopaque slow-growing tumor-like mass within skeletal muscles of primates.

8: ASSESSMENT OF PRECLINICAL DEVELOPMENT NEUROPATHOLOGY USING IMAGE ANALYSIS

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Neuropathological assessment of the effect of toxic compounds on the central nervous system requires classification and quantification of a variety of anatomical and histological endpoints. Current methods for calculating trophic effects in the brain

require time-consuming, manual measurements subject to considerable variability within and between raters. Automated deep learning-assisted digital image analysis provides an opportunity to decrease turnaround time for these studies and increase the precision of the data. This APP (Analysis Protocol Package) generates linear and area measurements of the neocortex, striatum, corpus callosum, hippocampus, and cerebellum. To improve reliability of the algorithm, the cortex was further segmented into the cingulate, motor, and somatosensory regions. Here, we show qualification data demonstrating the predictive strength and statistical power of automated methods for brain region segmentation relative to the manual measurements. The decreased variability of the automated methods improves the statistical power of the analyses and allows the APP to move closer to utilization in production, which will help pathologists complete Developmental Neurotoxicity (DNT) Studies with improved efficiency and precision and smaller sample sizes.

9: SEVERE VERMINOUS COLITIS CAUSED BY SPIROCERCA SP. IN FREE-RANGING GRAY FOXES (UROCYON CINEREOARGENTEUS)

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Background: Between 2017 and 2023, six gray foxes from California were presented for necropsy and diagnostic investigation due to suspected canine distemper virus infection. All showed intramural raised plaques in the colon with intralesional nematodes.

Objective: To describe the gross and histological lesions in the colon and morphologically and molecularly identify the nematodes found within the lesion.

Methods: A gross examination was performed on five whole gray fox cadavers and one collection of organs submitted from a gray fox. Formalin fixed, paraffin embedded sections of the colon were stained with hematoxylin and eosin, Masson's trichrome, Alcian blue, PAS, and Perls stain. Whole nematodes were collected in formalin and 70% alcohol and submitted for morphological and molecular identification.

Results: Histopathology showed focal to diffuse, moderate to severe, chronic, eosinophilic and histiocytic colitis with intralesional adult nematodes and free larvated eggs compatible with Spirurids (suspected to be *Spirocerca* sp.). Morphological identification classified the nematodes as belonging to the *Spirocerca* genus using measurements and characteristics of the buccal capsule and spicules. Molecular identification for the *Spirocerca* species is pending.

Conclusions: Spirocercosis has been described in other fox species such as red, Island and Andean foxes causing similar intramural nodular lesions in the esophagus, aorta, and stomach. However, colitis caused by *Spirocerca* sp. has not yet been reported in any carnivore, and spirocercosis has not been reported in the gray foxes. This report documents the pathological and parasitological finding of this nematode in six gray foxes with fatal CDV infection in California.

10: PECULIAR CLINICAL PRESENTATION AND DIAGNOSTIC RESULTS OF A CLASSIC CASE OF WIDELY METASTATIC CHOLANGIOCARCINOMA IN A SIBERIAN HUSKY DOG

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Background: A six-year-old spayed female Siberian Husky was evaluated for acute cervical pain and ataxia, respiratory changes, and a two-month history of weight loss. Antemortem diagnostics included complete physical and neurologic examination, CBC/serum chemistry, cervical and thoracic radiographs, abdominal ultrasound, and splenic and lymph node cytology. Abnormalities included C6-T2 myelopathy, mildly elevated hepatocellular enzymes, sternal lymphadenopathy, ill-defined hepatosplenic nodules, and multifocal lymphadenopathy with concern for metastatic carcinoma cytologically. Humane euthanasia was elected.

Methods: A diagnostic necropsy was performed. Tissues were formalin-fixed and routinely processed before H&E staining. Affected tissues were further evaluated by trichrome staining and immunolabeling for CK7.

Results: Expanding and compressing the left medial liver lobe was an infiltrative, tan-yellow umbilicated mass measuring 5.5x4.0x3.0cm, which extended into the parenchyma on cut section. The remainder of the hepatic parenchyma contained up to 100 additional similarly appearing intrahepatic masses measuring up to 4cm in diameter. Additional masses were observed grossly within the gallbladder, spleen, mesentery, urinary bladder, diaphragm, lungs, pleura, and sternal lymph nodes. Histology of the primary and metastatic lesions was consistent with cholangiocarcinoma with varying degrees of anaplasia, and a marked scirrhous response was confirmed with trichrome staining. Immunolabeling for CK7 was negative.

Conclusions: Cervical pain represents an atypical presentation for widely metastatic cholangiocarcinoma and is a suspected sequel of vertebral metastasis or embolic spinal disease. The primary hepatic mass was not captured by routine AUS, and immunolabeling of the primary lesion was negative for CK7 expression, highlighting challenges to antemortem diagnostics and poor cellular differentiation, respectively.

11: FIRST CYTOLOGIC DESCRIPTION OF OLIGODENDROGLIOMA WITH EMBRYONAL COMPONENT IN A DOG

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Background: Oligodendroglioma is the most common subtype of glioma in dogs. Typical cytologic features are poorly described but might include paired or rowed cellular alignment, round uniform eccentric nuclei, deep cytoplasmic basophilia and an interspersed chicken wire-like capillary network proliferation.

Objective: Describe the cytologic features of an oligodendroglioma with embryonal (primitive neuroectodermal tumor [PNET]-like) component in a dog.

Methods: A 10-year-old castrated male German Wirehaired Pointer was presented for acute onset of clustered seizures. Imaging, laboratory, microscopic and immunophenotypical evaluations were conducted.

Results: An MRI revealed a hemorrhagic, strongly contrast-enhancing left frontal lobe mass. Histopathology and cytology following craniotomy revealed a well-demarcated, unencapsulated, and densely cellular tumor. Cells were individualized to aggregated or rowing, round with high N:C ratios, and had immature stippled chromatin, occasional visible nucleoli, blue cytoplasm and moderate anisokaryosis. Homer-Wright rosette and “pseudorosette” formations, capillary structures with large vascular spaces and mitotic figures were often noted. The typical honeycomb cellular appearance and “chicken wire” capillary arrangement were absent. Histologic evidence of invasion included scattered neurons within the mass and one focus of neoplastic cells within the neuropil. A diffuse nuclear immunoreactivity for olig2 with immunohistochemistry was observed and a diagnosis of oligodendroglioma with embryonal (PNET-like) component, grade 3 was made. The patient was doing well at a 30-days recheck post-craniotomy visit, and no overt neurologic deficits were noted.

Conclusion: The cytologic features of canine oligodendrogliomas might be atypical and resemble a PNET-like appearance, more common in astrocytomas.

12: CHYLOUS ASCITES IN AN ADULT HORSE: A CASE REPORT

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Background: Chylous ascites (CA) is a common entity in different species, but not in equids. To the best of our knowledge, no cases have been reported among adult horses.

Objective: To report a case of CA in an adult horse.

Methods: A 31-year-old, Arabian gelding, was presented for colic evaluation.

Results: Peritoneal fluid analysis revealed increased total protein of 3.2 g/dL and triglycerides of 421 mg/dL (ratio to serum ≈ 11.4). The nucleated cell count was 5490 cells/ μ L, and predominated by non-degenerate neutrophils, with only occasional small lymphocytes. Lipid-laden macrophages and Sudan III-positive fat droplets in the background were also noted. Due to clinical deterioration, euthanasia was elected. Necropsy revealed severe ulcerative gastritis, enterocolitis, odontoclastic tooth resorption, renal infarction, jejunal hemomelasma and a large sessile necrotic omental lipoma. No typical causes of CA (eg, visceral neoplasia, lymphangiectasia, etc) were identified.

Conclusions: Even though lymphocytes did not predominate and a definitive etiology could not be determined, a high triglycerides content was consistent with CA. Since

triglycerides are not regularly measured in equine abdominal fluids, we are unsure if CA in this species is underrepresented. We speculate that, if lipomas somehow leak or rupture inside the abdomen, a lipid-rich fluid accumulation mimicking CA might ensue. Further research is warranted to determine the prevalence and pathophysiological mechanisms of CA in horses.

13: NASAL HISTIOCYTIC SARCOMA WITH CEREBRAL INFILTRATION IN A CAT: A CASE REPORT AND LITERATURE REVIEW

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An 8-year-old, spayed female, Domestic Shorthair cat was presented to the Louisiana State University Veterinary Teaching Hospital (LSU-VTH) for anorexia and 2-month history of unilateral nasal discharge and wheezing. During venipuncture, the animal became obtunded and developed [RS1] nystagmus, miosis, bradycardia, and hypothermia. The cat was humanely euthanized and sent to the Louisiana Animal Disease Diagnostic Laboratory (LADDL) for necropsy. On postmortem examination, a pale gray, firm, multilobular neoplasm obliterated [RS2] [JCM3] the right caudal nasal turbinates and extended into [RS4] the left nasal cavity. The cells composing this neoplasm were morphologically consistent with histiocytes and had infiltrated [RS5] the meninges and superficial cortex of the rostral regions of the cerebral olfactory lobes bilaterally. On immunohistochemistry, significant labelling [RS6] [JCM7] of neoplastic cells for Iba-1 and feline CD18 strongly supported histiocytic lineage; therefore, this mass was diagnosed as a nasal histiocytic sarcoma (HS). HS is uncommon in cats; it rarely arises from the nasal cavity. A literature search spanning the past 15 years identified 19 reports of feline HS, 2 of which were of suspected nasal origin. Both cases similarly included invasion through the cribriform plate into the olfactory lobes, with upper respiratory [RS8] signs (e.g. nasal discharge, sneezing) on initial presentation, however, only one case developed neurologic signs[RS9] . Primary nasal HS should be considered as a differential for nasal masses in cats, especially those with concurrent neurological abnormalities.

14: CLINICAL AND HISTOPATHOLOGIC CHARACTERIZATION OF CANINE SINONASAL HAMARTOMAS

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Background: Sinonasal hamartomas are rare, under-reported, non-neoplastic lesions with potential to cause severe respiratory symptoms in dogs. In humans, these are classified based on cellular components as respiratory epithelial adenomatoid hamartoma (REAH), chondro-osseous respiratory epithelial adenomatoid hamartomas (COREAH), seromucinous hamartoma (SH) or nasal chondromesenchymal hamartoma. Objective: To describe histological characteristics, clinical features, and outcomes of canine sinonasal hamartomas.

Methods: Nasal biopsy samples submitted to PennVet Diagnostic Laboratory from 24 dogs (2016-2023) with lesions characteristic of hamartomas were reviewed. Clinical data were collected from submission forms and surveys.

Results: Twenty-four cases included: 23 COREAHs (2 with SH foci) and 1 REAH. Patients ranged from 3 to 16 years (median 8, mean 8) with 10 spayed females, 12 castrated males, and 2 intact males across 18 breeds. Histologically, the respiratory epithelial-lined polypoid masses contained edematous stroma and glandular structures; SH had proliferation of seromucous glands and COREAHs contained chondro-osseous cores. Four COREAHs had evidence of concurrent angiomatous tissue, atypia, or polyps. Patients presented with epistaxis, decreased nostril airflow, nasal discharge, and sneezing. Computed tomography imaging (n=22) showed destructive masses causing turbinate lysis and erosion of the cribriform plate and orbit. Follow-up data (n=15) reported 10 dogs are alive, 4 were euthanized due to decreased quality of life (range 0-13 months post-biopsy), and 1 died from an unknown cause. Treatments varied from saline nebulization, antibiotics, steroids, cyberknife, and rhinotomy.

Conclusion: Canine sinonasal hamartomas are an important differential diagnosis for locally destructive and recurrent nasal masses that may require on-going treatment for symptom management.

15: A BACTERIA NAMED MORGAN

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Morganella morganii is an enteric bacteria and known opportunistic nosocomial pathogen with proven resistance to several classes of antibiotics, such as 1st and 2nd generation cephalosporins and macrolides. Virulence factors like urease and hemolysin have helped *M. Morganii* to colonize the urinary tract and infect wounds in humans.

Previously *M. Morganii* has been identified in samples taken from the lungs, pericardium and epicardium of dairy cattle, as well as the lungs of piglets. However, this is the first time *M. Morganii* has been identified in the lungs and pericardial sac of a pet dog. The samples were collected at post mortem and cultured from a dog who tested positive for Canine Parvovirus, prior to euthanasia. The combination of virulence factors, antibiotic resistance, and propensity for infection of immunocompromised individuals makes the identification of this pathogen in a companion animal especially concerning.

16: CD21+ SMALL B CELL LYMPHOMA IN AN APPROXIMATELY THIRTEEN-YEAR-OLD DOMESTIC SHORTHAIRED FELINE

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An approximately thirteen-year-old, female spayed Domestic Shorthair feline was reported to have a history of pleural effusion (pseudochylous; 1.5:1 choletriglycerides) and a mediastinal mass. Flow cytometry was performed on an aspirate at Colorado State University and was reported as CD21+ small cell lymphocytosis. A full necropsy was performed including gross and microscopic examination at the Michigan State

University Veterinary Diagnostic Laboratory. Gross examination revealed an 8cm x 2.5cm x 1.5cm soft mass in the mediastinal region that extended to the apex of the heart and was mottled dark red to white. Approximately 90mL of white to pink tinged, opaque fluid in the pleural cavity contained refractile droplets. Microscopically, the mediastinal mass contained a densely cellular, poorly demarcated, and infiltrative proliferation of monomorphic small to intermediate neoplastic lymphocytes arranged in sheets. The neoplastic cells were round, had scant to moderate amounts of pale eosinophilic cytoplasm, and distinct cell borders. This feline was diagnosed with mediastinal lymphoma, most likely CD21+ small B cell in origin, based on microscopic analysis and flow cytometry results. Most cases of small cell lymphoma in felines responds well to treatment utilizing current chemotherapy options such as COP (cyclophosphamide, vincristine, prednisolone or prednisone) or CHOP (cyclophosphamide, doxorubicin, vinca alkaloid, prednisolone or prednisone); however, this patient continued to deteriorate despite being placed on the chemotherapy protocol. This case report highlights the importance of differentiation between small cell mediastinal lymphoma versus thymoma in addition to chemotherapy protocols and corresponding survival times for CD21+ small B cell mediastinal lymphomas in felines.

17: TWO CASE REPORTS OF MYCOBACTERIAL ARTHRITIS IN A DOG AND A CAT

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Background: Acid-fast bacteria is a group of bacillary, intracellular, opportunistic, and zoonotic microorganisms including the *Mycobacterium* sp. and *Nocardia* sp. genera. Infections might affect multiple organs but arthritis is scarcely reported.

Objective: Describe two cases of mycobacterial infection (one confirmed and another presumptive) in companion animals affecting an uncommon anatomical site: the joints.

Methods: The patients (a DSH cat and Briard dog) were both mid-aged, castrated males and presented independently with a history of lameness, and a history of prolonged immunosuppressive therapy (IT) for auto-immune thrombocytopenia.

Results: Synovial fluid cytology (SFC) revealed marked suppurative inflammation. In the cat's sample, negatively stained intracellular filamentous bacilli were observed and positive for Ziehl-Neelsen staining. No organism was cultured; thus, a presumptive diagnosis of *Mycobacterium* sp. polyarthritis was made. No microorganisms were observed in the dog's fluid sample, but culture revealed growth of Gram positive filamentous bacteria and PCR confirmed *M. fortuitum*. The dog had also developed concurrent multifocal pyogranulomatous mycobacterial panniculitis and sepsis, but IT reduction and antimicrobial therapy cleared his infection after 6 months. The cat died 5 days post diagnosis.

Conclusions: SFC might be a valuable diagnostic tool in establishing mycobacterial infection as a differential among animals with suppurative arthritis especially among immunosuppressed animals. The dissemination route was presumed to be hematogenous, yet it is unclear which factors determined tissue predilection. Further

studies reassessing the monitoring protocols applied to animals under long-term IT are warranted.

18: APPROACH TO HISTOLOGIC CLASSIFICATION OF CHOLANGIOCARCINOMA AND LOOK-ALIKES: A RETROSPECTIVE STUDY

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Background: Cholangiocarcinoma (CCA) is an aggressive biliary neoplasm that affects a variety of species, including humans, dogs, cats, bears, ruminants, birds, and reptiles. CCA represents the second most common primary liver tumor in humans but literature on CCA in veterinary species exists primarily in sporadic case reports and in companion animals.

Objective: Tumor classification of CCA subtypes derived from human pathology is discussed and utilized to review histologic features of CCA in veterinary species. In this study, we review gross and histologic classification of CCA 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).

Results: Seventeen cases of CCA were identified in companion animals, laboratory animals, and zoo/wildlife (mammals, n=8; reptiles, n=6; avian, n=3). Cases were predominately intrahepatic and mass-forming (16/17), with an equal proportion of large (9/17) and small duct origin (8/17), and were well (9/17), moderately (7/17), or poorly differentiated (1/17). Metastases to various tissues including spleen, intestines, lung, heart, pancreas, kidney, and mesentery were identified in 5 of 17 cases. Six additional cases resembled CCA, but on histologic evaluation were identified as benign biliary neoplasms, neuroendocrine tumors, polycystic liver disease, or metastatic adenocarcinoma.

Conclusions: Documentation of pathological features of CCA across veterinary species may be useful to guide future diagnoses and better understanding of translationally relevant features and diagnostic criteria.

19: CHARACTERIZING CARDIAC COLLAGEN CONTENT IN A PRE-TRANSLATIONAL MOUSE MODEL OF OSTEOGENESIS IMPERFECTA (OIM)

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Background: Osteogenesis imperfecta (OI) is a heritable connective tissue disorder that results in reduced bone mineral density, bone deformity and fragility, and intrinsic muscle weakness. OI is most commonly caused by defects in type I collagen genes and has an incidence of 1:10,000 births. OI patients die 7-10 years earlier than healthy

counterparts, with cardiopulmonary complications as the second leading cause of death. Preliminary studies in the osteogenesis imperfecta murine (*oim*) model, a model of severe human type III OI due to a mutation in *Col1a2* gene, suggest gross differences between *oim* and wild-type (Wt) hearts.

Objective: The objective of the present study is to quantify the collagen content of *oim* and Wt age- and sex-matched hearts.

Methods: Fixed cross-sections of 4-month-old male and female Wt and *oim* hearts were stained with picosirius red and histological images were semi-quantitatively analyzed for fibrillar collagen using Image-J software. Collagen content will be quantified using the hydroxyproline content of hydrolyzed heart tissue. Hydroxyproline is used as an indirect measure of fibrillar collagen content.

Results: We hypothesize that *oim* mice will have reduced total collagen content in their hearts as compared to Wt littermates, with male mice more affected than females.

Conclusions: These findings would support the hypothesis that alterations in type I collagen in the extracellular matrix of the *oim* hearts contribute to cardiac dysfunction and potentially the development of cardiac disease resulting in the early death of OI patients.

20: EXPLORING THE ROLE OF CHILDHOOD OBESITY IN CANCER: STRUCTURAL CHANGES IN ADIPOSE TISSUE STROMA

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Background: In the United States, childhood obesity has reached epidemic levels. With the increase in incidence of colorectal cancer correlating to childhood obesity, an understanding of the underlying mechanisms between obesity and adipose tissue is needed to develop preventive and interventional treatment modalities for cancer. It is known that in obesity, structural and biochemical changes occur in adipose stroma cells (ASCs) and extracellular matrix (ECM) that mimic the tumor microenvironment. Given this evidence, it is predicted that juvenile obese and aged adipose tissues will share features such as ASC cellular aging and ECM fibrosis.

Hypothesis: Obesity results in premature aging of ASCs and this effect is irreversible with weight loss.

Methods: ASCs in this study were isolated via a diet-induced obesity (DIO) mouse model consisting of the following cohorts: lean/low fat diet (LFD) juvenile mice, obese/high fat diet (HFD) mice, formerly obese mice, and lean aged mice. For each ASC line, immunofluorescence microscopy was used to assess myofibroblast differentiation, cellular proliferation by BrdU incorporation, and ECM composition by fibronectin, collagen I and VI immunostaining.

Results: Myofibroblast differentiation is increased in the HFD and aged cell lines relative to lean and formerly obese cell lines. HFD and aged cells were less proliferative than other cell lines. Data for ECM composition is pending analysis.

Conclusions: The effects of obesity studied in this project appear to be reversible with weight loss. Aged lean ASCs were similar to obese ASCs isolated from younger mice.

21: EXOPHIALA SPINIFERA IN A CAT

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A male neutered adult domestic long-haired cat was brought to the North Carolina State University for a follow up examination regarding feline atopic skin syndrome. For the past year the cat was under treatment with cyclosporine and intermittent glucocorticoids. During the visit a large, soft, and ulcerated interdigital mass was seen on the inner aspect of the third toe of the right front paw. Cytologic examination of the mass revealed granulomatous inflammation with atypical round, blue-green and non-staining yeast-like organisms. Further investigation of the mass was performed by biphasic fungal culture leading to the initial assessment of fungal organism morphology to be *Sporothrix schenckii*, which was inconsistent with the cytologic appearance. However, on histopathologic examination, round pigmented fungal organisms were identified which resembled sclerotic bodies typically associated with chromoblastomycosis. Additional staining techniques including GMS and PAS were used and did not show characteristic internal septation of chromoblastomycosis. Instead, they showed rare budding, supporting a diagnosis of phaeohyphomycosis, another type of dematiaceous fungal infection. Subsequently, *Exophiala spinifera* was identified using PCR and DNA sequencing confirming a diagnosis of phaeohyphomycosis. Considering the timeline of the mass's appearance subsequent to cyclosporine and steroid therapy, raises suspicion that the infection stems from immunomodulatory treatment. This report highlights the necessity for molecular testing and interdisciplinary collaboration to accurately identify fungal agents, as illustrated by this case of misdiagnosed *Exophiala spinifera*. This case also emphasizes the challenges of identifying dematiaceous fungi such as *Exophiala spinifera* given its variable presentation.

22: MALDI-TOF MS METHOD TO IDENTIFY EQUINE LEUKOCYTES: FIRST STEP FOR DETECTION OF MELANOMA CIRCULATING TUMOR CELLS

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Melanocytic tumors are common skin neoplasms in horses. Although lesions tend to be benign and subcutaneously located, most tumors will progress to malignancy, infiltrating deeper into tissues, spreading to internal organs, and resulting in debilitating or fatal progression. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) uses a laser to ionize particles in order to detect peptide fingerprints for rapid identification of pathogens. The overarching goal of our study is to use MALDI-

TOF MS to detect circulating tumor cells (CTC) in cases of equine melanoma. Firstly, we established a library of equine blood leukocytes using 5×10^6 cells isolated from healthy horses. Proteins were extracted with formic acid and acetonitrile, using alpha-cyano-4-hydroxycinnamic acid as matrix, and mass spectra were acquired using a Bruker MALDI Biotyper sirius. This library was compared with leukocytes from sick horses (i.e., with abnormal proportions of leukocytes) and other species (e.g., pigs, dogs, cats, cows, and goats), and proved to be specific, correctly identifying leukocytes from healthy and sick horses but not from other species, although a minimum of 5×10^5 cells are needed. Samples can be stored for up to 72 hours and the intra-assay variability is $< 10\%$. Our future directions include using fewer cells to establish the library in an attempt to increase sensitivity, applying similar methodology to create a library of cultured normal and neoplastic melanocytes, and spiking blood samples with these cells to investigate the feasibility of MALDI-TOF MS as a diagnostic tool to identify melanoma CTC in horses.

23: DEVELOPMENT OF A CANINE LEUKOCYTE MALDI-TOF MS LIBRARY FOR FUTURE APPLICATION IN THE DETECTION OF CIRCULATING TUMOR CELLS

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Melanocytic neoplasms are commonly diagnosed in dogs. The malignant forms (i.e., melanomas) can release cells into the blood in the process of metastasis. Therefore, the identification of circulating tumor cells (CTC) can provide non-invasive and early detection of melanomas with metastatic potential, improving the prognosis of the affected animals. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an analytical technique that has been successful in identifying microorganisms and mammalian cells via protein fingerprinting. Given their unique melanin content, we aim to use melanoma as a model to investigate the feasibility of using MALDI-TOF MS in the detection of CTC of various neoplasms. Initially, we created a reference spectra library of canine leukocytes after their isolation and protein extraction using alpha-cyano-4-hydroxycinnamic acid as the matrix and a Bruker MALDI Biotyper sirius. We determined the lower limit of detection (1×10^5 cells), stability (up to 96 hours, either at 4 or -30°C), and intra-assay coefficient of variation ($< 10\%$), testing blood from both healthy and sick dogs. Future studies will focus on creating libraries for cultured normal and neoplastic melanocytes and use them to spike blood samples to determine the sensitivity of the method. The impact of this study is the generation of data to support future investigations to predict the metastatic potential, develop new therapeutic options, and assess treatment response in dogs with melanoma. Furthermore, it will develop the group's expertise to expand the use of the technique to other neoplastic diseases in domestic animals.

24: TONSILLAR CYTOLOGY OF THE DOG: A DESCRIPTIVE RETROSPECTIVE STUDY OF 32 CASES (2009-2023)

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Background: Diseases of the canine palatine tonsil include infection and primary or metastatic neoplasia, which are amenable to cytologic diagnosis. The literature on tonsillar cytology is limited primarily to case reports.

Objectives: This retrospective study describes diagnostic yield, cytologic characteristics, and diagnostic accuracy of canine tonsillar cytology.

Methods: Electronic medical record search retrieved 32 cases. Cytology slides were reviewed using a standardized process by a board-certified clinical pathologist and a veterinary student. A board-certified anatomic pathologist and a student reviewed corresponding histology (n=16). PARR was used to further evaluate lymphoid tissue in cytology samples (n=13).

Results: Diagnostic yield was 91%. Diagnostic agreement for the major pathologic process by cytology compared with histopathology was 75%. Concordant diagnoses were rendered for three lymphomas, four carcinomas, one inflammatory process, two lymphoid hyperplasias, and two cysts. Discordant diagnoses included three false negative cytologic diagnoses (two lymphomas and one carcinoma by histopathology) and one false positive lymphoma (lymphoid hyperplasia by histopathology). PARR correlated with the histologic evaluation of lymphoid tissue in all but one case and with all cytologic results lacking histology.

Conclusions: The quality of the submitted preparations was good with high diagnostic recovery rate. Common features of tonsillar cytology include: blood, lymphoid cell populations, inflammatory cells, and epithelial tissue. The concordance of cytologic and histologic interpretation of the major pathologic process was 75%; discordant results included three cases in which cytology missed malignant neoplasia and one case of a false positive diagnosis of neoplasia. PARR correlates well with microscopic impressions.

25: MONOCYTIC/AZUROPHILIC LEUKEMIA IN A BEARDED DRAGON (POGONA VITTICEPS)

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Leukemia is occasionally diagnosed in bearded dragons, with cases of chronic monocytic leukemia, acute myeloid leukemia, and lymphoid leukemia being reported. An adult intact female bearded dragon (*Pogona vitticeps*) of approximately eight years of age presented with a history of decreased appetite and depression of several days' duration. Upon physical examination, stringy saliva in the oral cavity (suggesting dehydration), pale and yellow-tinged mucous membranes, and forceful expiration were noted. Radiographs and clinical signs were consistent with pneumonia. The patient was treated with subcutaneous fluids and enrofloxacin. A hemogram revealed a normal

packed cell volume and marked leukocytosis, consisting primarily of medium to large round cells, supporting a diagnosis of acute leukemia. A few tumor cells contained low numbers of purple cytoplasmic granules. The patient died at home four days after presentation. On post-mortem examination, coelomic effusion and hemorrhages in multiple organs were identified. On histologic examination, there was splenic effacement with a neoplastic round cell infiltrate in perivascular and interstitial spaces and within blood vessel lumens of most organs, including the lungs. There was no apparent involvement of the bone marrow. The animal was tentatively diagnosed with disseminated round cell sarcoma and leukemia, pending further testing. Cytochemical staining of blood smears supported a monocytic/azurophilic origin for the tumor cells, leading to a final diagnosis of monocytic/azurophilic leukemia, potentially arising from the spleen. Using immunohistochemistry, low to moderate expression of Iba-1 was detected on immunohistochemical staining in 10-25% of the neoplastic cells, further supporting a monocytic/azurophilic cell origin.

26: METASTATIC MAMMARY CARCINOMA IN A SUGAR GLIDER (PETAURUS BREVICEPS)

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A 7-year-old, intact female, sugar glider (*Petaurus breviceps*) presented to the University of Illinois zoological medicine service for an acute onset of respiratory distress and lethargy. On physical examination, the sugar glider was tachypneic with crackles auscultated in all lung fields. Four months prior to presentation, a fine needle aspirate of a mammary mass was diagnostic for neoplasia of epithelial origin. Humane euthanasia was elected and necropsy requested. On gross examination, the patient was markedly cachexic. A large, solid, tan, subcutaneous mass was present within the right inguinal region and the adjacent marsupium oozed purulent material. Additional masses expanded the sublumbar and axillary lymph nodes, and multifocally effaced the lungs. Histologically, masses were composed of expansile nodules of neoplastic epithelial cells arranged in disorganized, crowded and piling sheets, nests, and glandular acini which often contained proteinaceous secretory material. Neoplastic cells had a high mitotic rate with bizarre mitoses, marked anisocytosis and anisokaryosis, and frequently invaded vessels. Mammary carcinomas are common tumors in domestic animals and have been reported in a variety of exotic species. Metastatic mammary carcinomas in sugar gliders have a relatively low prevalence in the population and are associated with very poor clinical outcomes. This case shows the clinical signs, gross, and histologic lesions associated with mammary carcinoma in a sugar glider.

27: METASTATIC OSTEOSARCOMA AND IRIDOPHOROMA IN AN ARGENTINE HORNED FROG (CERATOPHRYS ORNATA)

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Background and Objective: Reports of neoplastic diseases in Argentine horned frogs are few, and only hemangiosarcoma, lymphangioma, and iridophoroma have been documented. The clinical course and pathological findings of metastatic osteosarcoma and recurrent iridophoroma in this species are presented for the first time.

Materials and Methods: A 3-year-9-month-old, female, domestic Argentine horned frog presented with a left dorsal cutaneous mass that was surgically excised. The mass recurred nine months later, and second surgery was performed. The animal then showed paralysis of the right forelimb and a hard mass on the right shoulder region with obvious osteolysis. After the animal died, an autopsy was performed. Representative tissues were routinely processed for histopathology.

Results: Histologically, the cutaneous mass consisted of interlacing bundles of round to spindle-shaped cells. The neoplastic cells contained variable amounts of intracytoplasmic gold-brown to green, highly birefringent pigment, consistent with an iridophoroma. The right shoulder mass was composed of sheets of spindle to angular-shaped cells, with abundant production of osteoid and bone matrix. Osteosarcoma was diagnosed, and metastatic lesions were observed in systemic organs, including the heart and lungs.

Conclusions: This is the first description of iridophoroma and osteosarcoma in an Argentine horned frog. Iridophoromas are known to have malignant potential, so careful postoperative follow-up is needed. Similar to other species, the prognosis of osteosarcoma was quite poor, showing aggressive biological behavior in an Argentine horned frog. Accumulation of previously unreported diseases is crucial in diagnosing tumors in this unique domestic pet.

28: SARCOID IN A MUNTJAC DEER (MUNTIACUS REEVESI)

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A 15-year-old female Reeves Muntjac (*Muntiacus reevesi*) from a zoological collection was evaluated for a firm, pink, approximately 1 cm diameter pedunculated mass with focal mild ulceration on the right shoulder of 9 days duration. The mass was surgically excised and submitted for histopathology, which showed a well circumscribed, highly cellular neoplasm composed of spindle cells interspersed with bundles of disorganized collagen and pronounced epithelial rete pegs, suggestive of sarcoid. Fluorescent in situ hybridization for bovine papilloma virus (BPV) DNA was negative, but immunohistochemistry for BPV showed rare intranuclear intraepithelial positivity, and BPV PCR on formalin fixed paraffin embedded tissue returned positive. These findings support a diagnosis of BPV associated sarcoid. As of 7 months post diagnosis, the patient has not experienced local recurrence nor developed further masses, and the cohoused muntjac is healthy. Bovine papilloma virus is known to cause sarcoids in equids and fibropapillomas in cattle, but is uncommon in other species and has not been previously reported in muntjac. Sarcoid may be a rare but important differential diagnosis for cutaneous soft tissue tumors in exotic ruminants and this diagnosis has implications for biosecurity in zoologic collections. This case also demonstrates that multiple diagnostic tests may be necessary to corroborate a histologic suspicion of sarcoid.

29: CHARACTERIZATION OF POSTMORTEM TRAUMA AFTER FALL FROM HEIGHTS IN DOGS AND CATS

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After perpetrators commit acts of cruelty in the form of blunt-force trauma, they might conceal their acts by dropping the cadaver from heights, which causes additional lesions to the body. Hemorrhages allow the distinction between ante- and postmortem changes in fresh bodies but is much less useful in decomposed cadavers. Freshly euthanized puppies (n=10), adult dogs (n=10) and kittens (n=8) were separated into two groups and dropped from 40 feet and 80 feet respectively. All animals had pre-necropsy radiographs taken. Liver lacerations were present in 100% of animals. Of both species, 65% had skull fractures (puppies (93%) and kittens (50%)). Rib fractures were present in 80% of all dogs and mostly in puppies (76%). Although true pulverization of the solid inner organs was not observed, 27% of animals showed rupture of the kidney capsule, and 13% showed fragmentation of the liver into multiple, smaller, detached pieces. Limb fractures were present in 44% of cases, 42% of which were adult dogs and 2% were puppies. Fractures of the spine were seen in 17% of animals. Pre-necropsy radiographs were essential to the identification of most bone fractures. The histology of all fractures confirmed the absence of vital reaction or repair, with pointy sharp edges of the bone fragments typical of wet fractures. The distribution of these lesions is different from those seen in animals with high-rise syndrome, motor-vehicle trauma, and accidental or non-accidental injuries (NAI).

30: FOAL PERITONEAL FLUID ANALYSIS WITH MILK AND BIO-SPONGE INTERFERENCES

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A 2-day-old colt was presented to the University of Missouri Veterinary Health Center for evaluation of profuse watery diarrhea. Initial laboratory testing revealed acute inflammation and lactic acidosis. Fecal PCR testing for *Clostridium difficile* toxin A & B was positive. Treatment with IV fluids, antibiotics, an NSAID, Bio-Sponge (DTO smectite), and sucralfate yielded initial improvement. On day 2 of hospitalization the foal became increasingly uncomfortable (colicky), laboratory findings deteriorated, and abdominal effusion developed. Peritoneal fluid analysis on an ADVIA 2120i revealed a prominent discrepancy in the peroxidase and baso channel total nucleated cell counts, and lipid interference was visible on both cytograms. Microscopic evaluation identified 52.5% large mononuclear cells, often containing clear lipid vacuoles, 41.5% degenerate neutrophils, and 6% small lymphocytes. Coarse yellow refractile material was seen extracellularly and within inflammatory cells. No infectious organisms were found. The presence of lipid and refractile material in the peritoneal fluid were interpreted as milk and either Bio-Sponge or sucralfate, respectively, and indicated an upper gastrointestinal perforation. Microscopic evaluation of a suspension of Bio-Sponge was compatible with the coarse refractile debris observed in the patient's peritoneal fluid. The patient was subjected to euthanasia and necropsy revealed a duodenal perforation

with fibrinous peritonitis and severe colitis. Histopathology revealed coagulative necrosis of the duodenum and clostridial typhlocolitis. This case is an unusual example of concurrent milk and Bio-Sponge within equine peritoneal fluid and highlights the utility of cytogram assessment to investigate interferences in effusion samples.

31: MALIGNANT CANINE ORAL NEUROENDOCRINE TUMOR OF SUSPECTED CHEMODECTOMA ORIGIN

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Background: A 9.7-year-old intact male English bulldog presented to the University of Wisconsin-Madison Veterinary Care Radiation Oncology service for a heart-base mass consultation. Based on location, signalment, and CT imaging revealing cranial mediastinal lymphadenopathy, a malignant chemodectoma was suspected. Pertinent history includes right-sided congestive heart failure, recurrent pericardial effusion, and peritoneal effusion. An approximately 1-cm oral mass located in the left mandibular labial mucosa was incidentally identified on physical examination upon starting palliative radiation therapy and Palladia treatment for the heart-base mass.

Objective/Methods: To determine its etiology, the oral neoplasm was incompletely excised, evaluated histologically, and immunohistochemically stained for vimentin, synaptophysin, CD204, CD3, CD20, melanin-A, and pancytokeratin. Fontana-Masson and Giemsa stains were also performed. A CK20 stain and melanocytic tumor panel were also performed at referral laboratories. **Results:** Histologic analysis of the oral neoplasm showed packets of round to polygonal cells with frequent karyomegaly and vacuolated cytoplasm, marked pleomorphism, and epitheliotropism. The neoplastic cells were strongly immunopositive for vimentin (~90%) and synaptophysin (~70-80%). The Fontana-Masson, Giemsa, CD3, CD20, CD204, CK20, and pancytokeratin stains, and melanoma panel were negative.

Conclusion: Based on morphology and immunohistochemistry, this oral mass is most consistent with a malignant neuroendocrine tumor, potentially related to the suspected chemodectoma. Chemodectomas are most common in brachycephalic breeds and rarely metastasize, making the possible oral metastasis in this case diagnostically interesting and unexpected. Thus, this case demonstrates the need for comprehensive, full-body work-ups and highlights diagnostic strategies for poorly defined neoplasms.

32: ASSESSMENT OF DECALCIFICATION SOLUTIONS ON CELLULAR MORPHOLOGY AND IMMUNOSTAINING OF MOUSE BONES

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Introduction

Decalcification is a necessary step that allows hard, bony tissue samples to be trimmed,

sectioned, and stained. Some solutions act fast but damage proteins while others preserve proteins and morphology but take an excessive amount of time. This raises questions about what products can balance both resolution and processing time. Here, mouse tissues were decalcified with three commercially available solutions to identify optimal agents for diagnostic use that are time-efficient and cost-effective without compromising cellular detail and downstream immunochemistry results.

Materials & Methods

Immunocal (StatLab), Epredia decalcifying solution, and Rapid-Cal (StatLab) decalcification agents were tested on formalin fixed CD-1 mouse femur, skull, and sternum samples. Multiple metrics, including testing supernatants with ammonium oxalate and radiography, were used to assess various stages of bone demineralization. Tissues were routinely processed, embedded in paraffin, and sectioned at 5µm for H&E staining. Sections were also stained with markers for CD3, CD31 and IBA-1. Scoring was based on the degree of calcification (as indicated by radiolucency in x-ray, gross bone pliability, percent of mineralized tissue in slides, and histologic architecture preservation) at each timepoint (4, 6, 12, 24, 30, 48, 70 hours).

Results & Conclusion

Immunocal was most effective at decalcifying tissues under 48 hours without sacrificing sample quality based on gross and histologic scores. Epredia achieved comparable decalcification scores grossly but had moderate loss of cellular detail. Rapid-Cal slides scored poorly due to lack of comparable cellular detail. Immunohistochemistry staining resulted in appropriate labeling for all samples, regardless of decalcification solution.

33: ABERRANT LIVER FLUKE MIGRATION IN A MISSISSIPPI GOAT

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A 1.5-year-old female mixed breed goat presented to MSU-CVM Food Animal Service with a history of hindlimb ataxia. Treatment with anthelmintics, dexamethasone, and thiamine were unsuccessful. Humane euthanasia was elected. On gross examination, the right lateral midabdominal subcutaneous tissues and musculature contained a locally extensive black gelatinous area. Within the abdominal cavity, the omentum contained similar widely disseminated irregular black areas. The liver was covered in a mat of tan to black fibrinous material and was multifocally adhered to the diaphragm by areas of fibrosis. On cut surface, the liver had multifocal variably sized trematodes within black migration tracts. On cut surface of the caudodorsal lungs, there were similar tracts and trematodes. On histopathologic examination, the hepatic capsule was markedly thickened with black pigment and inflammation; fibrosis extended haphazardly through the hepatic parenchyma with associated inflammation and fluke exhaust. At the level of spinal cord at T10-T12, there was extensive white matter vacuolation, axonal degeneration, and multifocal Gitter cells. At T10, there was also fibrosis extending into the spinal cord with admixed black pigment and macrophages (consistent with fluke migration). Both *Fasciola hepatica* and *Fascioloides magna* are thought to be present in this case. *Fasciola hepatica* primarily causes acute disease in goats, a direct host. However, goats are an aberrant host of *Fascioloides magna* so massive tissue damage can occur. Both trematodes infect the host via consumption of metacercariae encysted

on vegetation or of the intermediate host, lymnaeid snails. Treatment using benzimidazole anthelmintics is recommended, but resistance grows yearly.

34: NECROHEMORRHAGIC PNEUMONIA IN TWO DOGS ASSOCIATED WITH CNF1+ HEMOLYTIC E. COLI

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Two unrelated dogs from different households, one 7-year-old male coonhound and one 12-year-old male neutered Standard Poodle, presented to MSU-CVM 6 months apart for postmortem examination following death with either no history or one limited to acute clinical signs including fever, tachypnea, and a moist cough. Upon gross examination, common findings included red-tinged mucoid nasal discharge and extensive pulmonary hemorrhage. Histopathologic findings were also similar, and these included abundant pulmonary hemorrhage, bacilli within alveolar macrophages, alveolar necrosis and thrombosis of pulmonary vessels. Bacterial culture was performed on lung in each case, and both yielded growth of hemolytic *Escherichia coli*. The *E. coli* isolates were positive for cytotoxic necrotizing factor 1 (CNF1) via PCR. While *E. coli* is ubiquitous in the environment and in the gastrointestinal tract of many animal species, some strains are pathogenic and cause either enteric or extraintestinal disease. Hemolytic strains expressing CNF1 are strongly associated with extraintestinal infections. While rare, extraintestinal *E. coli* should be considered in cases of acute necrohemorrhagic pneumonia.

35: CHARACTERISTICS ON PRESENTATION OF CLINICAL AND POSTMORTEM EXAMINATIONS IN GUINEA PIG LYMPHOMA, AND A REVIEW OF DIAGNOSTIC METHODS.

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Lymphoma is a commonly reported neoplastic disease in guinea pigs (*Cavia porcellus*) but is not typically diagnosed until it has progressed to high stage in malignancy. Overall, there is negligible literature on presentation and diagnostics for guinea pig lymphoma. We reviewed the clinical presentations and diagnostics from medical records of guinea pigs seen at the UW-Madison School of Veterinary Medicine Teaching Hospital between 2016-2022 that were diagnosed with lymphoma on necropsy. Two female and five male guinea pigs aged 2 to 5.9 years were included. Chief presentation complaints ranged from hyporexia to acute collapse. Death (3 cases) or euthanasia (4 cases) was due to acute death (2 cases), respiratory failure (3 cases), or neurologic disease (2 cases). Five guinea pigs had complete CBCs with WBC counts ranging from $11.2\text{--}441.9 \times 10^3/\mu\text{L}$ and lymphocyte counts $2.8\text{--}406.5 \times 10^3/\mu\text{L}$. Four guinea pigs underwent CT scans indicating generalized lymphadenopathy with other secondary changes suggestive of lymphoma. Four guinea pigs had fine needle lymph node aspirates which showed neoplastic lymphocytes (4). Radiographs in one guinea pig showed pulmonary metastatic disease. Necropsies revealed multicentric diffuse lymphoma via gross and histologic examination results with the organs most likely

affected being the lymph nodes (6/7), liver (7/7), spleen (7/7), lungs (6/7), and kidney (6/7). Immunohistochemistry indicated B cell origin (5 cases), T cell origin (1 case), or undetermined (1 case). We find that the correlation between clinical presentation of symptoms, antemortem diagnostic findings, and organ systems affected can help improve recommendations of preferential diagnostics pre-mortem.

36: BACTERIAL CONTAMINATION DOES NOT INFLUENCE THE DIAGNOSTIC ACCURACY OF URINE PROTEIN:CREATININE RATIO AND URINE SDS-PAGE

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Background: Urine samples received for testing commonly contain bacteria, and a recent study found that in dogs with urinary tract infections (UTI), protein banding patterns on urine sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were sometimes altered in urine with bacteriuria, even in the absence of hematuria and pyuria. Additionally, a previous study has demonstrated an elevated urine protein:creatinine ratio (UPC) in dogs with experimentally induced UTIs characterized by bacteriuria, pyuria, and hematuria. It is unclear to what extent bacteria and their proteins could contribute to the elevated UPC or SDS-PAGE banding patterns in these studies.

Objectives: To determine if the presence of bacteria or bacterial-produced proteins alter the UPC and contribute to the urine protein banding patterns observed with SDS-PAGE.

Methods: Aliquots of urine from ten clinically healthy, spayed/neutered dogs were inoculated with approximately 10^5 colony-forming units per milliliter (CFU/mL) of either *Escherichia coli*, *Staphylococcus pseudintermedius*, or *Proteus mirabilis* and incubated at room temperature (25°C) or 4°C for 2 and 5 days. After incubation, a quantitative urine culture was performed on each aliquot to determine the bacterial CFU/mL, and the urine supernatant from each condition was collected for UPC and urine SDS-PAGE.

Results: No significant increases in UPC and no alterations of banding pattern were observed for any bacteria or condition.

Conclusions: The diagnostic accuracy of urine SDS-PAGE or UPC are not expected to be altered by bacterial contamination.

37: JUVENILE DIABETES MELLITUS AND UNILATERAL RENAL AGENESIS IN A 6-MONTH-OLD KITTEN

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Background: Diabetes mellitus (DM) and chronic kidney disease (CKD) affect 1 to 3% of adult cats but are rarely reported in kittens. The pathogenesis of both conditions is yet to be fully understood. A link between these entities in people has been recently demonstrated.

Objective: Report a case of DM in a kitten with unilateral renal agenesis.

Methods: A 6-month-old castrated male, DSH kitten was presented for trouble breathing, polyuria and dehydration. The kitten had a history of urinary tract infection and iron deficiency anemia. Thorough laboratory and imaging diagnostics were conducted.

Results: The combination of clinical signs, marked hyperglycemia, elevated serum fructosamine, glucosuria, ketonuria, acidemia, renal azotemia and a serum osmolarity of 426 mOsm/L supported the diagnosis of diabetic ketoacidosis (DKA), hyperosmolar syndrome, and renal insult. Abdominal ultrasonography revealed the left kidney was absent. Euthanasia was elected. Post-mortem evaluation further revealed megaesophagus, hepatic lipidosis and pancreatitis with vacuolar degeneration. Pancreatic inflammation seemed chronic and vastly affecting the exocrine tissue portion, but immunophenotypical analysis revealed a robust population of islet beta cells remaining.

Conclusions: The development of DKA despite the absence of more archetypical endocrine-pancreatic lesions suggested this kitten was under type II (insulin-resistant) DM – the same kind people with CKD develop. It is unclear whether this kitten's insulin and renal dysfunction were related. Further research to investigate this correlation, and if cats with renal disease are predisposed to DKA, is warranted.

38: SELECTED RANGE, CALIBER, AND FIREARM TYPE AND THEIR EFFECTS ON GUNSHOT WOUND CHARACTERISTICS IN CANINE INTEGUMENT

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The characteristics of canine gunshot wounds (GSWs) and correlated distance estimates are missing from much of the medical literature. Therefore, veterinary pathologists must extrapolate what is known about human GSWs to guide their findings, which can have implications in court decisions in cases of animal abuse and cruelty. This study aimed to provide insight on GSWs to canine integument for clinical and diagnostic veterinarians. Four firearm calibers (12-gauge shotgun, .38 special handgun, .22 handgun, .22 rifle) were fired from three distances [0 inches (contact), 6 inches (close-range), 3 feet (intermediate-range)] to the head, chest, abdomen and hindlimb on euthanized canine cadavers (n=27) and on four different-colored canine hides. Dense fur coats and darker colors interfered with the gross examination. Contact and close-range entrance GSWs only caused soot deposition but no stippling nor tattooing as described in humans. Intermediate, entrance GSWs may be well hidden by long fur and only cause searing of the wound edges. While entrance wounds show bullet wipe, presence of bullet wipe on exit wounds is unpredictable. Gunshot-entrance wounds do have a somewhat different appearance on dogs' skin than on humans. The distance

evaluation is much less specific and less useful to shooting reconstructions. Growth in the veterinary forensics field not only holds weight in upholding animal welfare but also in detecting individuals who pose a threat to society.

39: INTEGRATING MEDICAL TWITTER AND COMPARATIVE PATHOLOGY: IS IT VIABLE?

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In the era of digitalization, different areas of science are utilizing social media in a variety of manners. Yet comparative pathology remains one of the more obscure areas in the biomedical research community. Twitter (Presently known as X) has around 350 million users worldwide that include a large community of biomedical researchers and medical professionals. Specifically, human pathologists are known for their use of Medical Twitter, where they discuss research, and diseases with colleagues, trainees, researchers and even patients. However, the comparative pathology community lacks a strong presence on the website. This may lead to gaps in knowledge and unawareness of the involvement that comparative pathologists' should have in biomedical and translational research. Here we assess the use of Twitter as a tool for collaboration, distribution and gathering of information in relation to the comparative pathology profession. This project utilizes Vanderbilt University Medical Center's (VUMC) Pathology Twitter page to share comparative pathology-related content through the Translational Pathology Shared Resource (TPSR) core facility, using #VUMCTPSR. These posts range from comparative histopathology, and collaborative publication highlights, to histotechnology tutorials. Since launching, we have reached up to 2000 views, with an average of 1000 user views [YTS1] per post utilizing various methods of social media engagement. Our goal is to showcase that through the appropriate use of social media, we are able to promote the importance of comparative pathology expertise amongst researchers utilizing animal models.

40: PATHOLOGICAL FEATURES OF EQUINE HERPESVIRUS TYPE 1 IN AN INFECTIOUS DISEASE IN BLACKBUCK (ANTILOPE CERVICAPRA)

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Background: Equine herpesvirus type 1 (EHV-1) causes abortions and respiratory disease in horses, whereas EHV-1 causes neuronal symptoms and death to non-equid species. EHV-1 is prevalent in non-equid species overseas but has not been confirmed in Japan. In one Japanese zoo, three blackbucks and a pregnant zebra died after indicating neurological symptoms.

Objective: This study aimed to clarify the pathological features of neuropathic infections caused by zebra-borne EHV-1 in blackbucks, including comparisons with EHV-1 infection in horses. These are the first cases of a lethal zebra-borne EHV-1 epidemic in

non-equid species in Japan, and the first case describing the pathogenicity in blackbucks.

Methods: Necropsy was performed on the three blackbucks and two zebras (adult and fetus). In addition, histopathological, virological, and autopsy imaging were analyzed for the two blackbucks and zebras.

Results: Lesions such as intranuclear inclusion bodies, neuronal necrosis, malacia, and edema of the neuropil were seen in the pons and piliform lobe in two blackbucks. In immunostaining, EHV-1 antigens were observed in the neurons and nasal epithelium. EHV-1 was detected in the central nervous system (CNS) by polymerase chain reaction (PCR) tests. Genome analysis using PCR amplifications showed that EHV-1 from the CNS was closely related to EHV-1 previously isolated from Onagers in the U. S. EHV-1 was not detected in the zebras by any methods.

Conclusions: Zebra-borne EHV-1 infectious disease was diagnosed in both blackbucks. The distribution of the lesions and viral antigens indicated that EHV-1 reached the brain via neurotropic pathways and created severe CNS lesions.

41: CUTANEOUS T-CELL LYMPHOMA IN A NAÏVE RAT

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Background: A 2-year-old, male intact, 646g, experimentally naive Long Evans rat was euthanized and submitted for necropsy due to clinical decline associated with craniofacial lesions and truncal excoriations.

Methods: A complete postmortem examination was performed. Tissue specimens were fixed in 10% neutral buffered formalin and processed for histopathology with hematoxylin and eosin. Immunohistochemistry for CD3, CD20, and IBA1 was performed on selected tissues.

Results: Postmortem examination revealed discoloration and swelling of the right muzzle, numerous abrasions and ulcers on the head and trunk, and a soft, moveable, multinodular, subcutaneous mass within the ventral neck. Histologically, the ulcers had extensive dermal to subcutaneous infiltrates of medium-sized lymphocytes with scant, lightly eosinophilic cytoplasm, round to ovoid nuclei with coarse chromatin, and rare mitoses. Neutrophils were rare despite ulceration. Intact craniofacial skin had intraepidermal aggregates of lymphocytes, and periadnexal, perivascular, and perineural lymphoid infiltrates. The tongue had similar aggregates in the mucosa, and deeper perivascular and perineural lymphoid infiltrates. The neck mass consisted of enlarged lymph nodes with infiltrates of similar lymphocytes. Immunostaining of the lymphoid infiltrates was positive for CD3 and negative for CD20. Plasma cells and IBA1+ cells were scattered within and beyond the infiltrates.

Conclusions: Histology and immunohistochemistry findings are consistent with cutaneous T-cell lymphoma, reports of which are rare in rats. To our knowledge, this is the first reported case of cutaneous lymphoma in a rat characterized by epitheliotropism and angiocentric and perineural infiltrates. This case shares features with mycosis fungoides and primary aggressive epidermotropic CD8+ T-cell lymphoma.

42: HISTOLOGIC CHARACTERIZATION OF BROOD X CICADAS

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Several ecological observational studies have been performed on periodical cicadas, but population histology has not been characterized. The aim of this project was to histologically characterize Brood X cicadas. We hypothesized that we would identify lesions contributing to mortality in cicadas found dead. Fifty-one Brood X cicadas (31 found dead (FD); 20 caught live (CL)) were collected in Powell, OH and examined. Gross features were used to differentiate between sex and the three species within Brood X (*Magicicada septendecim*, *M. cassini*, and *M. septendecula*). Males were overrepresented in the FD cicadas and underrepresented in the CL cicadas. Cicadas were fixed in Davidson's fixative for 5-13 days, trimmed using serial transverse and sagittal sections, then processed routinely for histologic evaluation. After initial evaluation, 16 FD cicadas were excluded due to severe postmortem autolysis. Tissues identified included: cuticle (35/35), epidermis (35/35), skeletal muscle (35/35), mouthparts (30/35), cephalic glands (35/35), tracheae and tracheoles (35/35), brain (34/35), peripheral nerves (33/35), compound eyes (35/35), foregut (33/35), epidermal glands (34/35), midgut (35/35), hindgut (23/35), fat body (34/35), Malpighian tubules (25/35), heart (9/35), aorta (28/35), vessels (33/35), and gonads (34/35). Minor lesions in the cuticle (8/15 FD; 12/20 CL), epidermis (2/15 FD; 12/20 CL), skeletal muscle (7/15 FD; 8/20 CL), and mouth parts (3/15 FD; 7/20 CL) were most common. Evidence of systemic disease (*Massospora cicadina* infection, n=2; bacterial sepsis, n=1; protozoal infection, n=1) was rare and only identified in CL cicadas. Bacterial/fungal overgrowth was common in FD cicadas, and causes of death were not apparent.

43: AGE-RELATED CHANGE IN ROUTINE CLINICOPATHOLOGICAL PARAMETERS AMONG APPARENTLY HEALTHY DOGS

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In the clinical setting, hematological and biochemical laboratory results are routinely utilized in diagnosing and treating dogs. It is vital that lab results are interpreted in the appropriate and accurate context. Misinterpretation can lead to misdiagnosis and/or incorrect therapeutics. Limited prior studies suggest that senior and geriatric dogs are likely to have some results outside the reference intervals on routine clinicopathologic tests, even when their owners consider them healthy. The objective of our study was to build upon previous studies by describing changes in clinicopathological parameters associated with aging in owner-reported "healthy" dogs. In the Dog Aging Project (DAP), dog owners enroll their pets by completing a web-based survey that collects information including owner-reported overall observations of health, as well as prior and ongoing medical conditions. A subset of these dogs have comprehensive biospecimens

collected annually. In the first collection year, 966 dogs had biospecimens collected and processed. Associations of parameters from CBC, biochemistry, and urinalysis with age were determined utilizing linear regression. Analysis of year one data demonstrated that in dogs with owner-reported “very good” or “excellent” health, and without any owner-reported ongoing chronic conditions, lymphocytes, USG, WBC, and BUN all decreased with age while globulin, ALP, platelets, and total protein all increased with age. These results suggest that although some clinicopathological parameters change as dogs grow older, these changes may not interfere with the quality of life or the healthspan of the dog and could indicate the presence of normal age-related change rather than onset of disease.

44: EVALUATING CHEMOKINE AND SPHINGOLIPID RECEPTOR EXPRESSION IN CANINE DIFFUSE LARGE B CELL LYMPHOMA (DLBCL).

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Introduction:

Lymphoma is the most common hematopoietic cancer in dogs. When treated with chemotherapy, canine lymphomas typically result in survival times of about one year. However, only a small percentage of patients experience long-term remission. Current prognostic methods based on immunophenotype or histological subtype are unable to accurately predict which patients will respond better to treatments. Therefore, there is a need for biomarkers to identify which patients are more likely to benefit from chemotherapy and achieve sustained cancer remission. In humans, the expression patterns of chemokine receptor CXCR4 and sphingolipid receptors S1PR1 and S1PR2 have been associated with prognosis in various forms of lymphoma. Hence, there is a possibility that the expression of chemokine and sphingolipid receptors could serve as prognostic indicators in canine lymphoma as well.

Objective:

Determine whether the most common form of canine lymphoma, DLBCL, expresses receptors CXCR4, S1PR1, and S1PR2.

Methods:

Immunohistochemistry using antibodies validated for canine receptors was performed on 86 archived cases of formalin-fixed and paraffin-embedded canine DLBCL. A rubric incorporating

staining intensity
and distribution scored immunoreactivity as weak or moderate/strong.

Results:

All receptors were detected in canine DLBCL. CXCR had 14.7% weak and 85.3% moderate/strong staining. S1PR1 had 37.7% weak and 62.4% moderate/ strong staining. S1PR2 had 75% weak and 25% moderate/strong staining.

Conclusion:

The variability in CXCR4, S1PR1, and S1PR2 staining across canine DLBCL cases suggests that these receptors might have utility as a prognostic biomarker. Prospective studies are necessary to correlate expression patterns with clinical outcomes in dogs with lymphoma.

45: DEVELOPMENT OF A DEEP-LEARNING ARTIFICIAL INTELLIGENCE SUPPORT TOOL FOR EVALUATING RAT KIDNEY

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The potential utility of digital pathology has become a subject of significant interest for both diagnostic and research pathology institutions. The Charles River Laboratories-Deciphex partnership develops digital and deep learning-enabled artificial intelligence (AI) tools that provide decision support for pathologists' evaluations of general toxicology studies. One of these diagnostic support tools or classifiers identifies normal and abnormal classes in rat kidney, targeting common microscopic findings observed in toxicology studies up to 3 months in duration. Normal classes consist of cortical tubules and interstitium, medullary tubules and collecting ducts, hilum, renal pelvis, glomeruli, and background. Abnormal classes include pigment, hyaline droplets, inflammatory infiltrates, tubular degeneration/necrosis, tubular dilation, tubular basophilia, and Chronic Progressive Nephropathy. The classifier was evaluated qualitatively (review of probability masks) and quantitatively (F scores and confusion matrices) by the trainer, experts (pathologists) and the classifier developers (Deciphex). Annotations to improve performance were made based on these reviews. As the classes demonstrated F scores of at least 0.7, the classifier was evaluated on novel (testing) slides. We continue to improve classifier performance with additional annotations and novel slide applications. Preliminary results indicate that our rat renal classifier using deep-learning artificial intelligence technology will be a useful diagnostic support tool in digital pathology.

46: A POST-MORTEM STUDY OF THE WILD EURASIAN OTTERS (*LUTRA LUTRA*) FOUND DEAD FROM 2015 TO 2020 IN THE SHETLAND ISLES, SCOTLAND, UNITED KINGDOM

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Post-mortem examinations provide important insights for the investigation of mortality, health status and demography of threatened species. The Eurasian otter (*Lutra lutra*) is a semi-aquatic carnivore, which is listed as Near Threatened (NT) under the IUCN red list. The aim of this study was to determine the geographical distribution of *L. lutra* deaths in the Shetland Isles, Scotland, in order to identify locations where improvements in road construction could be made to reduce deaths due to road traffic accidents. A total of 77 otters found dead in the Shetland Isles from 2015 to 2020 were collected and 16 of the otters underwent detailed dissection following a designed protocol for otters. Overall, there was no sex bias and the seasonal pattern of otters found dead differed over the years. Most specimens were found in spring (n=26), followed by winter (n=21). The distribution of collection sites was concentrated in the central region on the main road (A970) which extends between the north and south of the main island. Most of the otters were adults (70%, n=40) with good nutritional status. The major cause of death was blunt force trauma due to road traffic accidents, presenting with fractures, hemoabdomen and diaphragmatic hernia. In conclusion, based on the post-mortem findings, sites of high mortalities due to road traffic accidents have been identified, which may provide useful information for improvements in road design, such as warning signs and the provision of tunnels to allow otters to cross under roads.

47: METASTATIC TESTICULAR TUMOR IN A DOMESTIC DUCK

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Background: A domestic male intact duck was kept in a zoo enclosure and was reportedly unable to stand. This duck died acutely before veterinary care could be provided. This duck was submitted for necropsy to the Michigan State University (MSU) Veterinary Diagnostic Laboratory (VDL).

Methods: A full necropsy was performed by a board-certified poultry veterinarian (RMF) and representative samples of all major organs were collected for histopathologic examination using hematoxylin and eosin stains. Additionally, immunohistochemistry was conducted for Melan-A, neuron-specific enolase (NSE), and desmin.

Results: Gross examination revealed a left testicle that extensively effaced and replaced by a semi-firm, white to tan, 5 x 2.5 x 2.5 cm mass. The right testicle contained dozens of similar nodules measuring 2-4 mm. The liver contained dozens of white to tan, semi-firm nodules ranging from 0.5 to 1.5 cm. Severe emaciation was evident, characterized by muscular atrophy and the absence of subcutaneous or coelomic adipose tissue. Microscopically, the testicular mass consisted of rows of round to polygonal neoplastic cells within tubules supported by a dense fibrous stroma. The seminiferous tubules in

the contralateral testicle were devoid of spermatogonial cells and regionally replaced by a similar population of neoplastic cells. A similar population of neoplastic cells formed nodules within the liver. Immunolabeling was negative for Melan-A, neuron-specific enolase (NSE), and desmin.

Conclusions: Findings were consistent with a diagnosis of metastatic testicular seminoma. While many seminomas are generally benign in mammalian species, the extent of metastasis and/or malignant behavior in birds remains uncertain.

48: SYSTEMIC INFECTION DUE TO BOVINE ATADENOVIRUS F IN A CALF

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A 3-week-old female Highlander calf presented to the Texas A&M Large Animal Hospital recumbent and dehydrated. Over the next 36 hours, the calf was unresponsive to treatment, had periodic seizures, and died. Necropsy revealed mild icterus, tricavitary effusion, retrobulbar, perirenal and abomasal edema, necrohemorrhagic enterocolitis, and disseminated petechiae/ecchymoses on the subcutis, intestinal serosa, urinary bladder mucosa, spleen and lungs. The entire left cranial lung lobe was diffusely dark red and consolidated. A 10x4x5 cm hematoma was over the left parietal lobe. The lateral ventricles were moderately dilated (hydrocephalus). Double centrifugal sugar flotation from fecal contents revealed large numbers of *Strongyloides* sp. eggs. On histologic examination, widespread fibrinocellular thrombi within small caliber blood vessels associated with foci of hemorrhage and necrosis were found in the lymphoid and gastrointestinal organs. Intranuclear basophilic inclusion bodies were observed in endothelial cells of the renal glomeruli and in the adrenocortical cells along with necrotizing adrenalitis. Bronchopneumonia with intralesional foreign material was consistent with aspiration pneumonia. The respiratory viral panel for BRSV, BVD, PI3, BHV-1 and BCov was negative. *Salmonella* sp. PCR, *Clostridium perfringens* enterotoxin and *Clostridioides difficile* A&B toxins yielded negative results from the colon. The large intranuclear inclusion bodies were suggestive of an adenoviral infection. A pan-adenovirus PCR was performed and Bovine Atadenovirus F (former Bovine adenovirus serotype 7) was identified by sanger sequencing from formalin-fixed paraffin adrenal gland. This virus is endemic worldwide and primarily affects calves under one year of age, ranging from asymptomatic infections to severe enteric or respiratory disease.

49: PORCINE DERMATITIS AND NEPHROPATHY SYNDROME IN A 5-MONTH-OLD GILT

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A full necropsy was performed on a 5-month-old research gilt who was euthanized due to difficulty breathing, severe skin discoloration and scabbing. Routine gross and

histopathologic procedures were employed. On gross examination, there were myriad multifocal to coalescing, slightly raised, dark purple-black skin lesions along the lateral limbs, ventrum, and rump. Histologically, macrophages and lymphocytes surrounded and often infiltrated the walls of small and large vessels within the dermis, which were often occluded by fibrin thrombi. The surrounding dermis and subcutis were disrupted by copious hemorrhage. Similar lymphohistiocytic perivascularitis and vasculitis were present within the kidneys, heart, liver, esophagus, stomach, intestines, and pancreas. . Porcine circovirus-2 PCR on lung tissue was positive and porcine circovirus IHC on skin specimens had weak positive staining. Aerobic culture on skin and lung were negative, as were PCR tests for Porcine Reproductive and Respiratory Syndrome Virus, Porcine circovirus-3 and Swine Influenza Virus. Porcine Dermatitis and Nephropathy Syndrome (PDNS) is classically associated with porcine circovirus infection. The primary lesion is vasculitis leading to skin lesions, bilaterally enlarged and edematous kidneys, and dyspnea. Similar clinical signs and vascicentric pathologic lesions have been attributable to other swine viral and bacterial infections, indicating PDNS is a manifestation of systemic vasculitis and disseminated intravascular coagulation. This case shows the clinical signs, gross and histological lesions of PDNS in a 5-month-old gilt caused by porcine circovirus-2.

50: PRESENCE OF BALD EAGLE HEPACIVIRUS IN A SAMPLE POPULATION OF MICHIGAN BALD EAGLES

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Bald eagles, a culturally significant symbol in North America, were listed as threatened and endangered in the twentieth century due to illegal shooting, habitat destruction, and

environmental contamination. Conservation efforts and regulatory changes since then have facilitated their de-listing under the Endangered Species Act. Currently, the effect of infectious diseases on bald eagle population health has become a concern. As an example, a 2019 Wisconsin study associated a mortality event with a novel hepacivirus detected in bald eagles, termed Bald Eagle Hepacivirus (BeHV). Analysis of the viral genome identified similarities with the pathogen, hepatitis C virus, which causes cirrhosis in humans. The goal of our study was to determine if BeHV was present in tissue samples from n = 50 dead bald eagles submitted to the Michigan Department of Natural Resources in 2023 for postmortem examination and in n = 3 blood samples from live eagles. Two PCR assays targeting genomic regions encoding BeHV structural proteins were used to test fresh liver, fresh brain, and blood. BeHV viral RNA was detected in 38% (n = 20) of the eagles tested. Histopathologic examination of liver revealed mild lymphoplasmacytic inflammation in a subset of BeHV positive eagles; however, there was no significant association between inflammation and viral status.

Interestingly, chi square test revealed that significantly more adult bald eagles were BeHV positive relative to juveniles. This study is the first to identify the presence of BeHV in a population of bald eagles in Michigan. The clinical significance of BeHV infection remains unknown.

51: PATHOLOGIC CHARACTERIZATION OF ADENOVIRUS-CRE-INDUCED LIVER LESIONS IN TRANSGENIC ONCOPIGS

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Background: Comparable anatomy and physiology of pigs make them an ideal model for human diseases. Hepatocellular carcinoma reportedly may be induced in transgenic oncopigs with hepatic injection of adenovirus encoding Cre-recombinase (AdCre). AdCre activates mutant KRASG12D and TP53R167H genes, inducing tumors at the administration site. The original goal was to produce a hepatocellular carcinoma model within 4 weeks post-injection. Our objective was to characterize AdCre-induced lesions using multiplex immunofluorescence.

Method: Oncopigs (n=9) received hepatic AdCre injections and were sacrificed at 14 days (n=3), 21 days (n=3), and 28 days (n=3). Tissue microarray was created from FFPE liver sections and stained using a Leica Bond Rx autostainer and Akoya Biosciences Opal 7-color kit. Slides were imaged with Leica Versa 8 fluorescent scanner. Samples were evaluated for CD31 (endothelium), smooth muscle actin, CD45 (leukocytes), vimentin (myofibroblasts), Ki-67 (proliferation), and pan-cytokeratin (biliary hyperplasia). Halo v.3.6 was used for quantification and data was graphed and analyzed using GraphPad Prism v.9. A p-value of <0.05 was considered significant.

Results: By 4 weeks, lesions consisted predominantly of CD45+ leukocytes and vimentin. Ki-67+ co-localized with CD45+ cells consistent with immune cell proliferation. Vimentin and smooth muscle actin increased with co-expression in some areas consistent with myofibroblasts. Pan-cytokeratin variably increased in areas of reactive biliary hyperplasia.

Conclusion: Hepatic AdCre injection induces a strong inflammatory response in transgenic oncopigs, although the biological significance of such lesions as a tumor model remains unclear.

52: RETROSPECTIVE ANALYSIS OF RATTLESNAKE MORTALITY FROM 1983-2023

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Rattlesnakes are commonly kept in zoologic collections, with several species considered endangered in North America due to habitat loss and persecution. A retrospective study examining rattlesnake cases at the Michigan State Veterinary Diagnostic Lab (VDL) was conducted to contribute to our understanding of rattlesnake diseases. Necropsy reports including individuals in the genus *Crotalus* (n=45) or *Sistrurus* (n=21) or designated as “rattlesnake” (n=32) were collected via the MSU VDL

database. Ranging from 1983-2023, there were a total of 98 individual snakes examined of 13 different species submitted from zoologic collections. Causes of death were organized by the identified or suspected etiology. Of individuals with diagnoses, forty-four percent of the snakes had bacterial infections (n=36), and 17% of the snakes were diagnosed with neoplasia (n=14). Most systemic bacterial infections were caused by gram-negative bacteria (n=17), such as *Salmonella spp.*, but mycobacteriosis was also identified (n=4). The most common neoplasms identified were of mesenchymal origin (n=10), including soft tissue sarcomas, spindle cell sarcomas, and lymphosarcomas. Another notable condition in the examined snakes was fungal infection (n=8), which made up 10% of the diagnoses and primarily infected the skin (n=5). Understanding the spectrum of disease affecting these species is vital to their protection and management in captivity. These findings may guide husbandry and medical decisions in zoos that maintain populations for education and conservation.

53: UNLICENSED ANTIVIRAL PRODUCTS USED FOR THE AT-HOME TREATMENT OF FELINE INFECTIOUS PERITONITIS CONTAIN GS-441524 AT SIGNIFICANTLY DIFFERENT AMOUNTS THAN ADVERTISED

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Feline infectious peritonitis (FIP) is a fatal disease when left untreated and no FDA-approved therapies are available for veterinary use. This study analyzed the content of unlicensed GS-441524-like products being used as a largely successful at-home treatment for cats suspected to have FIP. This study analyzed the actual content of GS-441524 by liquid chromatography with tandem mass spectrometry (LC-MS/MS) of 127 injectable and oral samples from 30 of the most popular brands of unlicensed manufacturers. The remdesivir content and pH were also measured. Of the 87 injectable formulations, 95% contained more (on average 39% more) GS-441524 than expected based on the manufacturer's marketed concentrations. The average pH (1.30 pH) was well below the physiologic pH conditions recommended for subcutaneous injections. The oral formulations were somewhat more variable with 43% containing more GS-441524 (on average 75% more) than expected and 58% containing less (on average 39% less) than the expected content. There was minimal variability in GS-441524 content between replicate samples in the injectables (Coefficient of Variation (CV) < 10%) compared to the oral formulations (CV ranged between 1 and 32.5%). Some samples also unexpectedly contained remdesivir. All unlicensed products used for the at-home treatment of FIP that we tested contain GS-441524. The injectables generally contain significantly more drug than advertised at a below physiologic pH. Unlicensed oral products vary more widely in drug content and suffer from unconventional dosing and labeling. These data will inform future research and clinical use of at-home antiviral therapies for FIP.

54: HISTOLOGIC PARAMETERS IN CANINE EYELID MELANOCYTIC NEOPLASMS AND ASSOCIATION WITH KI67 INDEX

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BACKGROUND: Most cutaneous eyelid melanocytic neoplasms in the dog are classified as melanocytomas based on histologic prognostic parameters used for cutaneous melanocytic neoplasms in general. However, those parameters have not been investigated extensively or compared to Ki67 index specifically in canine eyelid melanocytic tumors.

OBJECTIVE: To compare histologic parameters (nuclear atypia, mitotic count, degree of pigmentation, ulceration, level of infiltration, tumor thickness) to Ki67 index in eyelid melanocytic neoplasms.

METHODS: We selected 120 cases of canine eyelid melanocytic neoplasms submitted to Michigan State University's Veterinary Diagnostic Laboratory for a prognostic melanoma panel. A Ki67 index of $\geq 15\%$ was used as the gold standard for diagnosing malignant melanoma. The above histologic parameters were retrospectively compared to the Ki67 index threshold of $\geq 15\%$ or $<15\%$ using Pearson's chi squared and Wilcoxon rank-sum tests.

RESULTS: The Ki67 index was $< 15\%$ for 92/120 (76.7%) tumors and $\geq 15\%$ for 28/120 (23.3%) tumors. Nuclear atypia ($p=0.001$), mitotic count ($p<0.001$), ulceration ($p=0.007$), and tumor extension beyond the dermis ($p=0.054$) were associated with Ki67 index. There was no significant association between Ki67 index and degree of pigmentation ($p=0.12$) or tumor thickness ($p=0.62$).

CONCLUSIONS: Similar to studies examining canine cutaneous melanocytic tumors at other locations, greater than 20% nuclear atypia, a mitotic count ≥ 3 , the presence of ulceration, and extension beyond the dermis are all associated with a Ki67 index $\geq 15\%$. A prognostic study with complete survival data would be necessary to further support the use of these parameters and the established threshold values.

55: MENINGIOMA OF THE LUMBAR SPINAL CANAL WITH GRANULAR CELL MORPHOLOGY IN A CAT

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An 8-year-old, female spayed, domestic shorthair cat presented to the University of Illinois neurology department with a two-week history of progressive weakness, hyporexia, and urinary and fecal incontinence. Lumbosacral magnetic resonance imaging revealed a 1.7 X 0.7 X 0.5 cm intradural oval-shaped mass from L4-L5 with severe spinal cord compression. The mass was sampled via fine needle aspiration, and cytology revealed a low number of rounded to spindloid cells with bright pink and granular cytoplasm, most suggestive of meningioma. The mass was surgically excised via a right-sided hemilaminectomy. Neoplastic cells on histopathology were haphazardly

arranged and spindloid to rarely rounded in shape with abundant eosinophilic and faintly granular cytoplasm. Cells were immunoreactive for both cytokeratin and vimentin, and the vast majority of the neoplastic cells were negative for PAS with rare individualized and rounded cells having diastase-resistant, PAS-positive granules. Histologic and immunohistochemical findings best supported a meningioma. A few months post-operatively a recheck MRI supported tumor regrowth. The patient was euthanized and postmortem examination revealed a segmental intradural mass from approximately L4-L7 with histologic findings confirming a regrowth of the originally diagnosed meningioma. Although granular cell meningiomas have not been included in the most recent WHO classification of brain tumors, this neoplasm displayed a granular cell phenotype lacking supportive histochemical evidence for granular cell origin. This case highlights the ambiguity regarding the differentiation of granular cell tumors and historically classified granular cell meningiomas.

56: CHARACTERIZATION OF LARGE DIFFUSE B CELL LYMPHOMA IN A CAPTIVE CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS*)

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Two cases of lymphosarcoma have been reported in California sea lions (*Zalophus californianus*), and the characterization and etiology of lymphosarcoma in this species is relatively unexplored. This report describes a large diffuse B cell lymphoma in a 30-year-old intact female California sea lion. The sea lion was raised in captivity and was euthanized due to quality-of-life concerns associated with aging. On necropsy, there was lymphadenomegaly of the mesenteric, ileoceocolic, and sublumbar lymph nodes, as well as masses in the spleen, liver, kidneys, and pancreas. Histologically, lymph node architecture was diffusely effaced and replaced by a poorly demarcated, unencapsulated infiltrative neoplastic large lymphocytic population. The renal interstitium, adrenal medulla, liver, and spleen were heavily infiltrated and replaced by sheets of neoplastic lymphocytes and fibrosis. Lymphocytic cells stained positively on immunohistochemistry for CD20 and CD79a and did not stain for CD3, consistent with a B cell lymphosarcoma. While neoplasms are relatively common in aged marine mammals, especially sea lions, this is the first reported case of a metastatic large diffuse B cell lymphoma in a captive sea lion. Continued examination of neoplasms, both viral and spontaneous, in these long-lived marine mammals can elucidate the mechanisms of neoplasia and reveal substantial differences in cancer mortality across mammalian species.

57: IMMUNOLABELING OF IBA-1, CD204, AND CD18 IN CANINE HISTIOCYTIC SARCOMA TISSUE MICROARRAYS

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Canine histiocytic sarcoma (HS) is a malignant hematopoietic neoplasm that can affect numerous organ systems. Most HSs originate from interstitial dendritic cells and are

classified as localized HS (single mass) or disseminated HS (multiple organs impacted). A less common type, hemophagocytic HS, originates from macrophages and manifests with erythrophagocytosis. The diagnosis of HS is aided by immunohistochemistry (IHC) for markers of cell lineage. Multiple IHC markers are used for classification but specificity and association with histological subtype are unclear. The objective of this study was to assess co-expression of the commonly detected antigens, Iba-1, CD204, and CD18, across the above three subtypes of HS to determine diagnostic sensitivity. Seventy-eight formalin-fixed paraffin-embedded samples previously diagnosed as HS by H&E staining (localized n=32, disseminated n=32, hemophagocytic n=14) were used to construct tissue microarrays (TMA) using two 0.6-mm diameter cores per tumour. IHC was performed for Iba-1, CD204, and CD18 on TMA sections and manual scoring was conducted. For disseminated, localized, and hemophagocytic HS, triple positive rates were 75%, 72%, and 86%, respectively. Double positive rates were 38%, 16%, and 7%, and single positive rates were 3%, 6%, and 0%. Only two cases, both localized, were triple negative. In this set of cases, most HSs were triple positive. More cases were positive for CD18 and Iba-1 (92 and 91%, respectively) than CD204 (83%), therefore either marker is more suitable than CD204 alone. Future studies will focus on greater definition of the specificity of these markers in non-malignant histiocytic diseases and non-HS neoplasms.

58: PULMONARY OCHROCONIS GALLOPAVA INFECTION IN A LAUGHING KOOKABURRA (*DACELO NOVAEGUINEAE*)

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A captive, 4-year-old intact male Laughing Kookaburra (*Dacelo novaeguineae*) was presented for significant exercise intolerance and weight loss of 6 months duration. Radiographs and computed tomography revealed increased soft tissue opacity within both lung fields. A complete blood count revealed heterophilia, monocytosis, and erythrocytosis. Clinical signs progressed despite empiric antibiotic and antifungal treatment, and euthanasia was elected. On gross necropsy, 95% of the lungs were tan to gray and friable. Histologically, the lung parenchyma was replaced by granulomas centered on myriad pale brown, 1-2 um-wide fungal hyphae with parallel walls, infrequent septation, and non-dichotomous branching. No other organisms were found in the lungs with Gomori methenamine silver stain, Gram stain, or Ziehl-Nielson acid-fast stain. Other histologic lesions included centrilobular hepatocellular necrosis and acute renal tubular necrosis, both of which were attributed to hypoxemia associated with the lung lesions. Fungal culture of fresh lung yielded *Ochroconis gallopava*. This pigmented fungal organism is ubiquitous in the environment, and infection has been described predominately within poultry and less commonly in exotic and wild birds. Ochroconosis in birds primarily localizes to the central nervous system, and less often to the lungs. In this case, lesions were confined to the lungs. This is the first reported case of *Ochroconis gallopava* infection in a Laughing Kookaburra. There are implications for zoological medicine as this fungal species has been increasingly recognized in captive wild birds.

59: CLINICAL AND HISTOPATHOLOGIC FEATURES OF FELINE GASTROINTESTINAL EOSINOPHILIC SCLEROSING FIBROPLASIA

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Background: Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia (FGESF) has been diagnosed in the alimentary tract and mesenteric lymph nodes of cats. Common clinical findings include eosinophilia and masses at the ileoceccocolic junction or pyloric sphincter. The cause and pathogenesis of FGESF is undetermined, but the presence of eosinophils suggests a genetic predisposition to eosinophilic inflammation or potential bacterial/parasitic infection. Previous studies have demonstrated mixed intralesional bacteria in FGESF and two cases have been associated with fungal hyphae.

Objective: In this study, we investigated common clinical, ultrasonographic, and histopathologic features of FGESF in 40 cases from multiple institutions.

Methods: Hematoxylin and eosin-stained sections of affected tissues were examined. Degree of eosinophilia, expansion of the submucosa and muscularis, eosinophils in tissue, degree of collagen, degree of necrosis, and presence of mast cells were determined for each section.

Results: Clinical data was available for 34 cases; all cats presented with gastrointestinal signs of varying duration with an average age of 9 years at presentation. Forty-five percent (n=22) of cats presented with eosinophilia. Histopathologic features of FGESF included thick trabeculae of collagen, eosinophils, plump fibroblasts, and mixed mononuclear inflammatory cells. The degree of eosinophilia, degree of expansion of the wall, or the number of eosinophils in tissue did not correlate with survival times. Mast cells were not considered a major feature in most of these cases.

Conclusions: The features investigated did not provide additional information for prognosis. Twenty-eight cases were treated surgically, 6 were treated medically, and 6 were diagnosed at necropsy.

60: BILATERAL GRANULOSA CELL TUMORS IN AN AFRICAN PYGMY HEDGEHOG (ATELERIX ALBIVENTRIS)

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A 1-year-old African Pygmy hedgehog (*Atelerix albiventris*) was presented for ovariohysterectomy and a history of hemorrhagic vaginal discharge. Gross pathology included a markedly thickened uterine body, and a vaginal vestibule moderately distended by blood-tinged fluid. Both the uterus and ovaries were examined via digital histology after routine fixation and hematoxylin and eosin staining. The ovaries showed occasional primary and secondary follicles, but the remaining ovarian parenchyma was effaced by nests and islands of neoplastic cells. These neoplastic cells were often arranged in pseudorosettes and had Call-Exner bodies within them which multifocally infiltrated adjacent ovarian stroma. Histology confirmed a diagnosis of bilateral granulosa cell tumors (GCTs). Sections of the uterus showed proliferation of the

endometrial mucosa with cystic degeneration and hyperplasia of endometrial glands, with occasional hyperplastic papillary projections extending into the uterine lumen. Adenomyosis was present along with notable inflammation transmurally. It is speculated that the GCTs produced higher levels of estrogen which resulted in endometrial cystic degeneration and hyperplasia with adenomyosis and endometritis. This pattern of pathology is often observed in dogs with GCTs and with similar uterine changes. More recently, Wu et al (2021) published GCTs in 8 African Pygmy hedgehogs, which showed the similar uterine changes observed in this case. Further histopathological investigation in pygmy hedgehog reproductive tissues when presented for routine ovariohysterectomy is needed to better understand the prevalence of this pathology among the captive population. Increasing awareness among primary clinicians and pathologists could improve diagnosis and treatment for this increasingly popular pet species.

61: DEVELOPING A GENOMICS WORKFLOW FOR FELINE HERPESVIRUS CLINICAL ISOLATES

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Feline herpesvirus type 1 (FHV-1), a double-stranded DNA virus, is a ubiquitous pathogen of felids. Although genetically stable, studies have reported sequence variation among FHV-1 isolates. Little is known about the genetic diversity within the UK, and no information exists on the phylogenetic relationship of UK strains. We aim to quantify the genomic diversity of FHV-1 within the UK and compare this with global FHV-1 sequences. To generate the required sequence dataset, we developed a laboratory workflow to prepare FHV-1 isolates for genomic sequencing on a Nanopore MinION sequencer. A set of forty-eight isolates derived from respiratory swabs submitted to the University's Veterinary Diagnostic Services was selected for analysis. Isolates were cultured through three passages in feline embryonic cells. Viral tissue culture fluid was clarified by centrifugation, filtration, and ultracentrifugation. DNA was extracted using a Taco Mini Automatic Extractor, and Nanopore libraries were prepared using the Rapid Barcoding kit. Our workflow generated sufficient concentration and purity of viral DNA to enable genomic sequencing of FHV-1 isolates with minimal host contamination. *De novo* assembly of the genome produced two contigs representing approximately 90% of the FHV-1 genome. When aligned to a reference sequence, the gap between the contigs was found to be located in a repeat region of the genome. A set of single nucleotide polymorphisms was identified. We have demonstrated that a culture/purification protocol is effective in generating FHV-1 DNA preparations which may be sequenced using Nanopore technology. This methodology will facilitate large-scale molecular epidemiological studies on FHV-1 diversity.

62: MALIGNANT AORTIC BODY CHEMODECTOMAS IN TWO DOGS

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The first case is a male neutered 12-year-old Miniature Schnauzer that was presented to MSU-CVM Emergency Services for a consult following a diagnosis of chemodectoma leading to increased respiratory rate and effort. After nonresponse to treatment, he was euthanized. On gross examination, there was a 5x5x3cm firm, multilobulated mass at the base of the heart and two 1x1x1cm white, firm, raised nodules on the epicardium of the right ventricle. Two additional similar nodules were found within the right ventricular wall. The adrenal glands were bilaterally enlarged. A 1.5x1.5x1cm purple, multilobulated, firm mass was in the jejunal mesentery. The right kidney had a 1x1x1cm, raised, white, firm nodule on the surface, and the left kidney had a 0.5x0.5x0.5cm similar nodule. Histopathology showed a malignant aortic body chemodectoma (aortic body carcinoma) of the heart base with metastasis to the right ventricle, mesenteric lymph node, lung, kidneys, adrenals, and brain.

The second case is a 9-year-old male neutered English Bulldog that was presented to MSU-CVM Diagnostic Lab for postmortem examination. On gross examination, there was a 9.5x9x3.5cm brown to red multilobulated mass at the base of the heart surrounding the aorta. A focal umbilicated tan to yellow transmural mass measuring 1.5x1.5x1cm was noted in the gallbladder wall. Histopathology showed a malignant aortic body chemodectoma (aortic body carcinoma) of the heart base with metastasis to the gallbladder.

Chemodectomas are slow-growing tumors that impact less than 1% of dogs and are generally benign. These cases represent uncommon instances of malignant chemodectomas with metastases.

63: POST-MORTEM EXAMINATION AND FINDINGS IN WILD EURASIAN OTTERS (LUTRA LUTRA): LESIONS ASSOCIATED WITH CAUSE OF DEATH AND INCIDENTAL FINDINGS

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Post-mortem examinations of specimens found dead provide important insights for the long-term management and conservation of wildlife species, particularly for the diagnosis and investigation of mortality and infectious diseases. The Eurasian otter (*Lutra lutra*) is an elusive semi-aquatic carnivore that is a sentinel species listed as Near Threatened (NT) under the IUCN red list and vulnerable in Scotland. Since the last published post-mortem study on Eurasian otters in Scotland was dated 2009, the aim of the current study was to describe gross post-mortem findings in Eurasian otters found dead in the Shetland Isles, Scotland, to provide an updated baseline record. Following a designed post-mortem protocol, the gross lesions presented were extensive and multifocal, reflecting the severity of the injuries. The cause of death included blunt force trauma, drowning and starvation. Foot lesions were the most common incidental findings. These findings in the current study will be presented as a brief photographic

reference as a diagnostic tool for the post-mortem examination of wild Eurasian otters. On-going mortality studies are required to allow further insights into the natural causes of death and long-term population trends for the Eurasian otters.

64: INTRAOCULAR METASTATIC CARCINOMAS IN CATS – A RETROSPECTIVE CASE SERIES FROM 2019-2023

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Carcinomas have been reported to metastasize to the globe, specifically the uvea due to its high vascularity; however, the histologic features of intraocular metastatic carcinomas in cats are not well-documented. This case series aims to systematically characterize intraocular metastatic carcinomas in cats, with a focus on features of presumed pulmonary carcinomas, that were submitted to the Comparative Ocular Pathology Laboratory Of Wisconsin (COPLOW) from 2019-2023. Submission forms, reports, and histopathology slides were retrieved from the COPLOW archive and reviewed. Cases with a histologic diagnosis of a metastatic intraocular carcinoma suggestive of pulmonary origin were included. Alcian blue stains and thyroid transcription factor-1 (TTF-1) immunohistochemical stains were also evaluated. Follow-up information was gathered via email and phone surveys. Eleven cases met inclusion criteria. No significant sex or breed predispositions were identified. Uveitis, glaucoma, and the presence of an ocular mass were the most common clinical ocular presentations. 10/11 cases had a thoracic mass identified by radiographs prior to enucleation and 3/10 cases had presumed metastatic lung disease. All neoplasms had epithelial arrangements, 8/11 cases exhibited carpeting behavior, and the anterior uvea was affected in 9/11 cases. Goblet cells were identified with Alcian blue staining in 4/11 cases. Neoplastic cells expressed TTF-1 in 2/11 cases. None of the cases with goblet cells were TTF-1 positive. The average survival time after enucleation was 34 days (9 cases). Ocular metastatic neoplasms can cause clinical uveitis and glaucoma, and once diagnosed, patients have a short survival time.

65: INTRA-ATRIAL NEMATODIASIS IN JOHNNY DARTERS (ETHEOSTOMA NIGRUM) IN COLORADO

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Background:

Fish can be used as sentinels of environmental health. Rare sino-atrial nematodes were incidentally recognized during a health survey of 1304 wild-caught Colorado Johnny Darters (*Etheostoma nigrum*) conducted in 2020-2021.

Objective:

To describe atrial nematodes found in Johnny Darters collected from the Saint Vrain and Big Thompson rivers.

Methods:

Fish and morphometric data were collected by the U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit as described previously.

Results:

Two fish had adult nematodes in the atrium and/or sinus venosus; one had a nematode in the pericardial space associated with hemopericardium. Nematode profiles were up to 275-370 microns in diameter with a smooth 2.6-3.9µm cuticle, pseudocoelom, lateral chords, coelomyarian musculature and digestive tract lined by columnar cells with a brush border and a simple esophagus. Testis was evident in one nematode profile. One of three fish had vasculitis of the sinus venosus with a mixed inflammatory infiltrate and fibroplasia. True prevalence of atrial nematodiasis may be higher if infection leads to a dramatically decreased survivability in affected fish. Future work may include next generation sequencing to identify the nematode genus and species, and identify potential life cycle and true or intermediate hosts. Additionally, further sampling to determine incidence in other water bodies and in relation to environmental parameters is indicated.

66: RIGHT ATRIOVENTRICULAR VALVE DYSPLASIA AND PULMONIC STENOSIS IN A DOG

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A 2-year-old spayed female Shih Tzu with a two-month history of exercise intolerance, weight loss, and anorexia was euthanized and submitted for necropsy after an acute episode of respiratory distress. Clinical examination identified a grade V/VI continuous heart murmur, and point-of-care ultrasound showed an enlarged right atrium, a reduction in cardiac contractility, and biventricular effusion. Postmortem examination revealed fusion of the chordae tendineae and thickening of the nonseptal valve cusp. Further cardiac findings included pulmonic stenosis with severe poststenotic pulmonary artery dilatation, right ventricular concentric hypertrophy, and two right ventricular aneurysms. Additionally, there was ascites and severe hepatomegaly. Histologically, the stratum spongiosum of the right atrioventricular valve was expanded by a myxomatous matrix arranged in prominent folds. The myocardium had multifocal myocardial fibrosis and foci of severe thinning and fibrosis of the right ventricular myocardium (corresponding to the aneurysms). Based on clinical, postmortem, and histologic findings, a diagnosis of right atrioventricular valve dysplasia, pulmonic stenosis, and right-sided congestive heart failure was made. Right atrioventricular valve dysplasia is an uncommon congenital heart defect in dogs, reported to represent 3 % of congenital heart disease cases in dogs, whereas pulmonic stenosis is more common and is reported to represent 32 % of cases. It is not uncommon to find multiple concurrent congenital malformations in dogs, and a genetic component has been reported with both pulmonic stenosis and tricuspid valve dysplasia.

67: RETROSPECTIVE EVALUATION OF SQUAMOUS CELL CARCINOMA IN NON-DOMESTIC FELIDS

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Squamous cell carcinoma (SCC), and its association with feline papillomavirus and/or UV light exposure, is well-documented in domestic cats, but little has been reported in non-domestic felids. Here we describe 14 cases of SCC diagnosed in non-domestic felids at the University of Tennessee College of Veterinary Medicine. Animals most commonly initially presented for evaluation of a primary tumor (12/14), with 2/14 cases identified only at necropsy. Affected species included tigers (9/14), lions (4/14), and a snow leopard (1/14). The average age affected was 16.6 years. No gender predisposition was identified. Cutaneous tumors arose from the ventral abdomen (4/15), eyelid (2/15), tail (1/15), digit (1/15), hindlimb (1/15), and pinna (1/15); additional primary tumors arose from the oral mucosa (3/15), tongue (1/15), and lung (1/15). Cutaneous tumors were typically ulcerated (13/15). Common histologic features were keratin formation (16/16), intratumoral inflammation (15/16), and a scirrhous response (13/16). Metastasis was identified in 6/14 cases, affecting the lymph nodes (5/6), lungs (3/6), liver (3/6), small intestines (2/6), spleen (1/6), adrenal gland (1/6), kidney (1/6), stomach (1/6), and peritoneum (1/6). Average survival time after diagnosis was 7.4 months; the most common cause of death was euthanasia due to local and/or metastatic SCC (8/14). Papillomavirus was not detected by PCR on formalin-fixed tissue in any sample (0/19 samples; 0/14 cases). SCC is important to consider in non-domestic felids due to negative impact on lifespan. Unlike in domestic cats, papillomavirus infection and UV light exposure do not appear to be predisposing factors in non-domestic felids.

68: PRESCOTTELLA (RHODOCOCCLUS) EQUI INFECTION IN A DOG WITH SEVERE PYOGRANULOMATOUS DERMATITIS, PANNICULITIS, AND LYMPHADENITIS

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A 5-month-old male intact bulldog was presented to a local veterinary clinic with a chronic ulcerative multinodular skin disease in the right inguinal region and inner thigh, and bilaterally enlarged popliteal, mandibular, and prescapular lymph nodes. Biopsies of the haired inguinal skin and right popliteal lymph node were submitted to the University of Missouri, Veterinary Medical Diagnostic Laboratory. Microscopically, there was diffuse severe pyogranulomatous dermatitis, panniculitis, and lymphadenitis with large number of foamy macrophages containing coccobacilli bacteria. The bacteria were both Gram-positive and acid-fast (Fite)-positive. The results were strongly suggestive of a *Prescottella* (formerly *Rhodococcus*) *equi* infection. Following nucleic acid extraction and real-time PCR using specific primers and probe, *P. equi* infection was confirmed. *P. equi* is a common etiology of granulomatous pneumonia and lymphadenitis in foals. However, it is sometimes observed in other animals, usually when immunocompromised. Cutaneous infection of *P. equi* has been reported in several cats

but not in dogs. The role played by specific virulent factors in cutaneous versus systemic disease caused by *P. equi* is currently unknown.

69: TERTIARY LYMPHOID STRUCTURES IN CANINE SOFT TISSUE SARCOMAS: CHARACTERIZATION AND EFFECT ON PROGNOSIS

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Background

Tertiary lymphoid structures (TLS) are transient microenvironmental aggregates of immune cells occurring in sites of chronic inflammation, including cancer, external to secondary lymphoid tissue. They are associated with improved prognosis in human sarcomas, which have similar features to canine soft tissue sarcomas (STS).

Objective

1) evaluate the prevalence of TLS within soft-tissue sarcomas; 2) characterize their RNA and protein expression; and 3) their effect on prognosis in canines with STS.

Methods

RNA expression in lymphoid aggregates and adjacent tumor tissue were measured in laser-capture microdissected FFPE tissue and compared to curl-derived RNA from control tissues. Marker expression was quantified using immunohistochemistry and digital image analysis. A retrospective study evaluated prognosis using data derived from the medical records of STS submitted through the CSU Veterinary Diagnostic Laboratory.

Results

The prevalence of tumors with at least one suspected TLS was 36 percent. B cells were concentrated in lymphoid aggregates compared with adjacent sarcoma tissue. Plasma cells and high endothelial venules, important markers of TLS, were seen within aggregate-containing tumors but not in control sarcomas. Survival times and recurrence did differ between TLS-containing and non-TLS groups.

Conclusions

We conclude that the lymphoid aggregates found within canine STS are compatible with TLS. Future work on this project aims to describe the immune contexture within TLS with the hopes of determining whether the immune environment is susceptible to immunotherapies related to checkpoint inhibition.

70: PITUITARY CORTICOTROPH ADENOMA IN A CAPTIVE ROCK HYRAX (*PROCAVIA CAPENSIS*)

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The rock hyrax (*Procapra capensis*) is a medium-sized terrestrial mammal found in Africa and the Middle East. An 8-year-old male captive rock hyrax was found dead with no reported antemortem clinical abnormalities. Postmortem examination revealed a large pituitary mass that compressed the overlying hypothalamus. Histopathology of the

pituitary gland revealed a circumscribed, minimally encapsulated, and expansile neoplasm composed of cords of neoplastic epithelial cells, consistent with chromophobes of the anterior pituitary gland. Immunohistochemical staining revealed scattered positive cytoplasmic staining for ACTH, findings consistent with a corticotroph adenoma. Given the absence of clinical and histopathological findings consistent with hyperadrenocorticism, this neoplasm was presumed to be non-functional. Other postmortem findings included locally extensive compression, malacia, gliosis, and perivascular hemorrhage of the overlying hypothalamus secondary to the space occupying nature of the neoplasm. This rock hyrax also had acute aspiration pneumonia and bacterial septicemia, interpreted as the proximal cause of death. While neurological abnormalities were not noted antemortem, microscopic lesions in the brain suggest that compression by the mass and subsequent neurological impairment could have been a risk factor for aspiration in this animal. Chronic hemosiderosis and cardiomyopathy were also noted, and are commonly reported in captive rock hyrax. Spontaneous neoplasia has been infrequently documented in rock hyraxes, and this case report adds to the list of neoplasms reported in this species.

71: PATHOLOGY OF NATURAL INFECTION WITH HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (H5N1) CLADE 2.3.4.4b. IN TWO WILD STRIPED SKUNKS

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Eleven wild striped skunks (*Mephitis mephitis*) were found dead on one property in Wisconsin over four days in October 2022, and two adults were necropsied. Nasal swabs and samples of brain, lung, and liver from both skunks were strongly positive for highly pathogenic avian influenza virus (HPAI) using a real-time PCR assay targeting HPAI H5N1 clade 2.3.4.4b. In contrast to other mammals infected with HPAI, histopathological lesions in the brain were minimal to absent in these skunks. Rather, primary findings were necrotizing interstitial pneumonia and randomly distributed foci of acute hepatic necrosis. The presence of significant lung pathology in conjunction with high viral load in the respiratory tract as detected by PCR suggest that the respiratory system may be an important site of HPAI viral replication in this species. Previous research indicates that skunks can shed high levels of low pathogenicity avian influenza virus through nasal and oral secretions. These findings suggest that skunks may be of concern for further HPAI virus dissemination in mammals. Skunks are common in peri-urban regions and the 11 skunks from this outbreak were noted to share provided food sources with domestic cats and domestic dogs on this property. Further research is warranted to evaluate the capacity of striped skunks to transmit HPAI between themselves or animals. In summary, these cases highlight pathologic features of natural infections with HPAI in skunks and reinforce that HPAI should be considered as a differential for acute mortality events in this species.

72: METASTATIC PHEOCHROMOCYTOMA IN A MINIATURE DACHSHUND

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An 8-year-old male neutered miniature Dachshund presented to MSU-CVM Internal Medicine Service and was diagnosed with a left adrenal mass that invaded the caudal vena cava. Histopathologic evaluation of the mass revealed an unencapsulated, well demarcated multilobulated neoplasm composed of polygonal cells arranged in nests and packets within a fibrovascular stroma, confirming pheochromocytoma. Four years later, the patient presented for abdominal distension, and multiple masses were identified on imaging. A fine needle aspirate was taken from the spleen and aspirated cells characterized by cohesive clusters, consistent with neoplastic cells. Due to poor prognosis the dog was euthanized. On postmortem examination there were coalescing perirenal masses that infiltrated and obstructed the lumen of the caudal vena cava, extended caudally into the large vessels of the hindlimbs, and cranially into the thoracic vena cava. The masses extended proximally into the spine, obliterated the body of L7, compressed the spinal cord, and infiltrated the epaxial muscles of the caudal lumbar and sacral region. The spleen had a 6.5x3.5x3cm mass on the head and three small dark red nodules on the tail. Histopathology confirmed a malignant pheochromocytoma with metastasis to the vena cava, L7, sacral epaxial muscles, kidney, liver, lung, spleen, pancreas, thoracic cavity, and femoral bone marrow. Pheochromocytomas often present as malignant neoplasms with local invasion of the vena cava and metastasis to distant organs. Of particular interest in this case is the prolonged four year interval between adrenalectomy and re-presentation and extreme local invasion into the spine and soft tissues.

73: DETERMINATION OF BACKYARD CHICKEN PLASMA PROTEIN REFERENCE DATA USING PROTEIN ELECTROPHORESIS

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Background: The existing literature concerning biochemical reference data in domestic chickens has predominantly concentrated on commercial flocks, overlooking backyard chickens. Recently biochemical reference intervals were established for non-commercial flocks in Colorado. However, the dye-binding method employed by biochemistry analyzers for measuring albumin cannot accurately determine albumin in low concentrations.

Objective: To ensure veterinarians are equipped to serve the growing number of backyard flocks, this study aimed to develop a population-based reference intervals for plasma protein biochemistry measurements in backyard chickens (*Gallus gallus domesticus*) using protein electrophoresis.

Methods: Heparinized blood samples were collected between May and July of 2023 from 150 non-commercial adult chickens that were deemed healthy and not in molt from 7 different privately owned backyard flocks in Colorado. Heparinized plasma samples

were analyzed on a Cobas c501 analyzer to determine total protein and a mammalian albumin kit using the bromocresol green method. The globulin fraction and A/G ratio were calculated. The samples were then analyzed using agarose gel protein electrophoresis to determine albumin, globulin fractions, and the A/G ratio. Reference intervals were generated using Microsoft Excel (Microsoft Office 2016; Microsoft) and the Reference Value Advisor Software, according to ASVCP guidelines for each method of analysis and the results were compared.

Results: Non-Partitioned reference intervals for electrophoretic fractions were generated using non-parametric methods and were: Albumin=1.39-2.95 g/dL, Alpha 1=0.13-0.32 g/dL, Alpha 2=0.33-0.91 g/dL, Beta=0.55-2.79 g/dL, Beta 1=0.28-1.4 g/dL, Beta 2=0.33-1.80, Gamma=0.25-1.29 g/dL.

Conclusion: This study established plasma protein electrophoresis RIs for backyard chickens in Colorado, which can be utilized.

74: QUANTITATIVE FLOW CYTOMETRIC MONITORING FOR REMISSION STATUS IN CANINE B CELL LYMPHOMA

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Background: B-cell lymphoma (BCL) is the most common hematologic malignancy of dogs. Eighty percent will relapse within one year of diagnosis despite initial complete response to chemotherapy. Flow cytometry is routinely used in the diagnosis of lymphoma, and has promise as a measure of objective response.

Objective: To characterize remission status in peripheral lymph node aspirates from dogs with BCL using flow cytometry.

Methods: Peripheral lymph node aspirates from dogs with newly diagnosed BCL were analyzed by flow cytometry prior to, and monthly, during chemotherapy until relapse. Standard RECIST criteria were used for response assessment. Samples with a medium-large size B cell population comprising more than 1% of nucleated cells were defined as positive for lymphoma. Time to clinical remission (TCR) and time to relapse (TTR) were measured from chemotherapy initiation.

Results/Conclusion: Thirteen dogs with BCL were included. Across all dogs, neoplastic B-cells represented 24%, 5.7% and 37% of cells at enrollment, completion of chemotherapy and relapse, respectively. CD25 expression was found on 51.3%, 7.1% and 63.6% of neoplastic B-cells at enrollment, completion of chemotherapy and relapse, respectively. TCR and TTR were 78 and 202 days, respectively. In 10 dogs, there was an increase in CD25 expression and medium-large B-cell percentage preceding relapse. Neoplastic B-cell percentage at diagnosis was not associated with TCR and

TTR. There was weak correlation between the percentage of CD25+ B-cells at diagnosis and TTR. Further work is warranted to incorporate the use of flow cytometry as a monitoring tool during chemotherapy for BCL.

75: PATHOLOGY OF RATTUS RATTUS AND RATTUS NORVEGICUS IN THE EASTERN CARIBBEAN

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Few studies have looked at pathology of wild rats, specifically *Rattus rattus* (the common black rat) and *Rattus norvegicus* (the common brown rat), which are the species of rats in the Federation of Saint Kitts and Nevis. This study aims to add to the general information on wild rat pathologies as well as provide a study on rats in a tropical climate. 102 rats were trapped from rural and urban areas including crop production areas and livestock facilities. They were euthanized and then autopsied. Histopathology was performed on collected organs (20 per rat). Overall, the organs with most lesions were the liver (73/102), followed by the lungs (49/102), heart (35/102), and kidneys (34/102). The most common change throughout all tissues was lymphoid hyperplasia in the spleen (93/102), lymph nodes (79/102) and MALT (85/102) followed by interstitial lymphoplasmacytic infiltration (in multiple organs), neutrophilic inflammation (in the lungs, heart, liver, skeletal muscles, salivary glands, and large intestines), fibrosis (in the lung, kidney, heart, liver, and skeletal muscles) and histiocytic infiltration (in the lungs, heart, and liver). Gastrointestinal parasites were a frequent finding with *Hymenolepis* (21/102), *Gongylonema* (74/102), and *Moniliformis moniliformis* (22/102) being the most common. Liver cysts containing larvae of *Taenia taeniaformis* were found in 34/102 rats. It appears that the immune system is stimulated and works well in response to the increase parasite burden in our rats. This study has helped create a baseline for studies on wild rats in Saint Kitts as well as the Caribbean.

76: SIMILARITIES IN GENE EXPRESSION INCLUDING SPEARMAN CORRELATION DISPLAYS RESEMBLANCE OF SPONTANEOUS CANINE ATOPIC DERMATITIS TO HUMAN ATOPIC DERMATITIS

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Mouse models for atopic dermatitis (AD) are commonly used for preclinical research; these do not reproduce the complexity of the human disease. Dogs naturally develop AD with similarities clinically and immunologically to their human counterpart. However, large-scale transcriptomic studies evaluating global gene expression patterns and inflammatory pathways in canine AD have not been reported. We characterized the atopic lesional (AL, 62 samples) and non-lesional (ANL, 29 samples) skin transcriptome in 33 AD dogs; 20 site-matching healthy skin samples from 12 dogs served as controls. Total RNA was extracted from skin biopsies and the transcriptome analysed using 150 paired-end RNA sequencing. The comparison of mRNA expression of spontaneous

canine AL and ANL skin with healthy skin identified 5,259 and 1,711 differentially expressed genes (DEGs) at ± 1.5 -fold change (P -adjusted value < 0.05) respectively. Top upregulated DEGs in AL and ANL skin were antimicrobial calcium-binding proteins *S100A12* and *S100A9*, keratinocyte gene *KRT2*, matrix metalloprotease *MMP12*, chemokines (*CCL5*, *CCL17*, *CCL22*, *CXCL10*) and interleukins (*IL*)-8, *IL*-13, *IL*-26 and *IL*-36G; these genes expressed higher fold changes in AL skin in addition to significantly upregulated *IL*-9, *IL*-17A, *IL*-17C, *IL*-22 and *IL*-24. Spearman correlation analysis utilizing a previously published human AD RNA-seq dataset revealed canine AL skin contained 6,475 shared DEGs (44%) with human AD (significant strong positive correlation $\rho = 0.57$, $p < 2.2 \times 10^{-16}$). In conclusion, spontaneous canine AD skin exhibits a striking similarity to human AD skin with multipolar axis T helper cell gene markers and including Spearman correlation and offers a promising research alternative to mouse AD models.

77: AN INTRAABDOMINAL ENDODERMAL CONGENITAL CYST IN A BOXER PUPPY

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A 6-month-old, cryptorchid male, Boxer presented for a 3-month history of abdominal distention, weight loss, and no vomiting. Abdominocentesis was performed where 1 liter of fluid was extracted and submitted for analysis with non-conclusive results. Two weeks following abdominocentesis, the animal returned with recurrence of abdominal distention. At this time, exploratory surgery was performed, and a large fluid-filled cyst was found in the abdominal cavity attached to the viscera. Punch biopsy of the fluid filled cystic cavity was taken and sent out for histopathology. Histopathology results concluded that the cystic mass was lined by simple cuboidal to columnar epithelium with some goblet cell differentiation, but the origin was not elucidated. Two months later, a second exploratory surgery was scheduled, and a larger cystic mass, approximately 3 liters, was found. The mass was deemed inoperable, and consequently euthanasia was elected. A postmortem examination revealed a large fluid-filled cyst was found to be 27cm x 35cm and occupying most of the abdominal cavity. The cyst was softly attached to the small intestines and the liver without invasion of the organs. The cyst wall was from 1cm to 3cm thick, with bosselated, intraluminal projections. Histopathology revealed a smooth muscle wall lined by simple cuboidal epithelium with occasional nests of glandular differentiation. The macroscopic and histopathological findings support the interpretation of congenital intraabdominal endodermal cyst. This is a rare mesenteric developmental lesion that needs to be included in a differential when young animals present with a distended abdomen and fluid accumulation.

78: CONIDIOBOLOMYCOSIS IN A QUARTER MARE HORSE

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An 8-year-old Quarter Horse mare presented to Mississippi State University College of Veterinary Medicine for a 3-month history of respiratory noise and bilateral mucoid to

serosanguinous nasal discharge. Radiographs showed a smoothly marginated, rounded tissue opaque mass measuring approximately 4 cm that caused deviation of the nasal septum. The larynx had an irregularly marginated, rounded, soft tissue opaque mass measuring approximately 4.7 x 4.0 cm. On endoscopy, multiple raised, red to orange, irregularly shaped, roughly marginated plaques were found in both nasal passages, which extended to the level of the ethmoid turbinates. A tissue sample measuring 1.8 x 1.2 x 0.7 cm from the rostral left nasal passage was taken for histopathology and fungal culture. Histopathology revealed moderate to severe eosinophilic rhinitis with epithelial hyperplasia, granulation tissue, hemorrhage, necrosis, and a rare hyphal profile. The hyphal profile was poorly stained with irregular walls. Microscopic evaluation of the fungal culture showed conidiophores with spherical conidia consistent with *Conidiobolus coronatus*. Panfungal PCR and sequencing confirmed *C. coronatus*. *C. coronatus*, a saprophytic fungus, is the most common upper respiratory fungal pathogen in horses. As in this case, small biopsies may have rare organisms and definitive diagnosis of *C. coronatus* is via fungal culture and/or PCR.

79: ATYPICAL CLINICAL PRESENTATION OF OPHIDIOMYCOSIS IN A FREE-RANGING EASTERN MILK SNAKE (*LAMPROPELTIS TRAIINGULUM TRAIINGULUM*) IN WISCONSIN

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An adult female Eastern milk snake (*Lampropeltis traingulum traingulum*; EMS) was found in a residential home. Grossly, retained shed was found on the left side of the face and small sections of the belly and sides, as well as degloving on the snout, and damaged scales along the body; there was no overt dermatitis. Mild stomatitis was found on presentation that progressed within one week to a white, friable mass on the glottis. Histologically, there were dermal and subcutaneous, well-demarcated, partially encapsulated granulomas throughout the head. Neighboring subcutis was expanded by mixed inflammatory infiltrates of heterophils, macrophages, and lymphocytes with fibrin deposition. Inflammation extended into underlying skeletal muscle, bone, and subspectacular spaces with associated degeneration and necrosis. In the oral cavity, there was ulceration of the oral and pharyngeal mucosa. Grocott's methenamine silver stain indicated fungal hyphae and arthroconidia characteristic of *Ophidiomyces ophidiicola* (Oo), which was confirmed by PCR of glottal tissue. Oo is the confirmed causative agent of snake fungal disease, which threatens snake populations. Oo has been detected in certain declining free ranging snake populations. Oo has only been reported in EMS captured in Wisconsin, Northern Michigan, and New York. This is the second PCR-confirmed case of Oo in an EMS in Wisconsin, which infrequently presents without initial dermatitis. The reoccurrence of Oo in the local EMS population suggests

that further surveillance is warranted to assess the risk to the population and due to the variation in clinical presentation.

80: CHRONIC LYMPHOCYTIC LEUKEMIA AND LYMPHOMA IN 19-YR-OLD ARABIAN MARE

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Lymphoproliferative neoplastic disorders are infrequently reported in equids. This case of multicentric lymphoma increases our understanding of this disease process in this species. A 19-yr-old Arabian mare, presented with progressive lethargy and decreasing appetite over a 4-week period. Antemortem abdominal ultrasonography revealed abnormal liver echotexture that was multifocally heterogenous and hyperechoic throughout the parenchyma. There was no imaging evidence of effusions, or other significant abnormalities. Antemortem bloodwork revealed marked leukocytosis further characterized by small, well-differentiated lymphocytosis and marked neutrophilia as well as severely elevated liver enzymes; all of which persisted for at least 5 days. Given poor prognosis due to concerns for lymphoid neoplasia, she was euthanized.

Significant findings at necropsy included diffuse hepatomegaly, splenomegaly, thickened glandular gastric mucosa, perirenal nodules, and lymphadenomegaly of multiple lymph nodes. A post-mortem bone marrow impression smear had numerous lymphocytes arranged in large clumps.

Histopathologically, multiple organs (kidney, liver, spleen, stomach, large and small intestine) were effaced by neoplastic small lymphocytes. The perirenal nodules were confirmed lymph nodes effaced by similar cells. Immunohistochemistry for CD3, CD79a, and CD20 further characterized neoplastic cells as a small T cell subtype. Additionally, severe submassive hepatocellular necrosis with bridging fibrosis and hemorrhage was observed in the liver and was attributed to neoplastic invasion.

Overall, both clinical and postmortem findings are consistent with late stage multicentric small T cell lymphoma with a leukemia. Further differentiation between primary lymphoma and primary leukemia is a diagnostic challenge in this case and similar lymphoproliferative disease in equids.

81: APPENDICEAL ADENOCARCINOMA PDX MODELS HAVE IMPROVED TUMOR GROWTH IN AN ORTHOTOPIC TUMOR ENVIRONMENT

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Background: Appendiceal Adenocarcinoma (AA) is a rare cancer that commonly metastasizes to the peritoneal cavity. Few preclinical models of AA exist. Patient-derived xenograft (PDX) models maintain molecular and histologic features of human tumors along with intratumoral heterogeneity. We hypothesized that orthotopic engraftment in the peritoneal cavity would more faithfully recapitulate the tumor microenvironment in AA.

Methods: Three AA PDX tumor models were used to compare tumor growth in the peritoneum and subcutis. Tumor growth rates were calculated and normalized. H&E and Immunohistochemical (IHC) staining were performed. Ki-67 staining was used to evaluate cell proliferation. Serial sections were stained with Ku80, CDX2, and vimentin. Slides were scanned with an Aperio AT2 whole slide digital scanner. Images were deconvoluted and merged using HALO v3.6 to evaluate human tumor cells and human stroma in PDX tumors.

Results: 2/3 of the tumor models evaluated showed a faster growth rate in the peritoneal cavity with increased Ki-67 proliferation rate and cellularity after engraftment. CDX2 and KU80 co-localized indicating that human AA cells persisted in both environments; however, the stroma was murine in both tumors.

Conclusion: Our results showed that AA PDX tumors implanted in the peritoneum have a faster growth rate, increased cell proliferation, and increased cellularity compared to tumors grown subcutaneously in 2/3 models evaluated. Also, PDX tumor cells persist in both environments but differences in the microenvironments murine stroma may impact tumor growth. This study supports our hypothesis that the peritoneal cavity is a preferred microenvironment for AA to engraft, grow, and metastasize.

82: FUSARIUM SPP. THE UNSEEN THREAT TO FLORIDA'S LEATHERBACK (DERMOCHELYS CORIACEA) POPULATION

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All leatherback populations are considered endangered, but some isolated subpopulations of leatherbacks are at higher risk of extinction. One major challenge facing leatherbacks is poor hatchling success. The cause of low hatchling success in leatherbacks is likely multifactorial but a link between decreased hatching success and the presence of *Fusarium* spp. in the nest has been made in all 7 species of sea turtles. The *Fusarium solani* species complex is known to cause sea turtle egg fusariosis (STEF), which causes death in sea turtle eggs. The causative agents of STEF are also known to cause mycotic dermatitis in leatherback sea turtle neonates. Using fungal cultures of sand and skin samples from leatherback nests and neonates we aim to relate the detection of *Fusarium* spp. across these samples to the development of mycotic dermatitis in post hatchlings, emergence success, and incubation temperature. We found *Fusarium* spp. in 40% of nest sand samples at excavation and on the skin of 18% of apparently healthy post hatchlings. We identified a relationship between the development of mycotic dermatitis in post hatchlings and low nest emergence success. Additionally, we found that nests with *Fusarium* spp. detected in one or more samples spent more time above 32°C. We concluded that *Fusarium* spp. are present in leatherback sea turtle nests in Florida and are affecting neonatal leatherbacks even after emergence. These data provide initial information to begin assessing hatchling

health and possibly elucidate a cause for decreased emergence success and survival in hatchlings incubated at higher temperatures.

83: POSTPARTUM CLOSTRIDIAL METRITIS DUE TO PAENICLOSTRIDIUM SORDELLII IN A NIGERIAN DWARF DOE

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A 4-year-old Nigerian Dwarf doe was presented to the Louisiana State University Veterinary Teaching Hospital for suspected metritis with a history of lethargy, anorexia, decreased milk production, and recumbency after kidding. CBC and venous blood gas analysis revealed marked neutropenia, hypoglycemia, metabolic acidosis, hyperkalemia, hypernatremia, and hyperchloremia. Despite treatment with insulin, dextrose, plasma, bicarbonate, and a synthetic plasma expander, the doe continued to decline and was humanely euthanized. On postmortem examination, the uterus had superficial to transmural dark gray to purple areas in both the caruncular and intervening regions, and reddish-brown, malodorous content. Histologically, the affected areas consisted of coagulative necrosis colonized by large gram-positive bacilli accompanied by suppurative inflammatory infiltrate. The goat also had mild neutrophilic splenitis. Immunohistochemical staining for multiple clostridial agents on uterine sections indicated the intralesional bacilli to be *Paeniclostridium sordellii*. Gross, histologic, and clinical findings in this case are consistent with previous reports of clostridial metritis and terminal exotoxemia/sepsis in goats, most commonly associated with retained placental remnants. *P. sordellii* has been reported to cause gas gangrene in ruminants, umbilical infections in foals, and metritis with septic shock in humans. Considering this report and the recently described cases of metritis caused by *P. sordellii* in goats, this microorganism should be considered a possible cause of postpartum metritis in does.

84: CAUSES OF MORTALITY IN CHAMELEONS IN WISCONSIN: A RETROSPECTIVE STUDY 2017-2023

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This retrospective study aimed to identify the causes of mortality reported in archived postmortem examinations and clinical records between 2017-2023 in veiled (*Chameleo calyptratus*) and panther (*Furcifer pardalis*) chameleons owned by clients of University of Wisconsin Veterinary Care and the Milwaukee County Zoo. Records from a total of 10 individuals were included, five Veiled, three Panther, and two unspecified breeds of chameleons. The ages of the individuals ranged from 5 months to 4 years. Causes of death were divided into five categories: neoplastic, nutritional, reproductive, respiratory, and gastrointestinal. Nutritional was the most common category, with the causes of

death including severe gout (1), hypervitaminosis D (1), hypovitaminosis E and/or selenium deficiency (1), and secondary metabolic disturbances due to severe dehydration (1). Neoplasia was the second most common cause of death with one case each of squamous cell carcinoma and metastatic chromatophoroma. The reproductive, respiratory, and gastrointestinal categories each consisted of one report each. Nutritional causes of death affected predominantly (3/4 cases) individuals less than 1 year of age, with the youngest affected being 5 months old. Clinical records indicate appropriate husbandry practices were reported in the majority of cases (3/4); nutritional imbalances were often suspected to be due to formulations of commercial multivitamin supplements. All other categories of causes of death affected individuals over 1 year of age. These findings indicate that husbandry practices still account for a large proportion of mortality in chameleons.

85: INVESTIGATING THE CD47 - SIRP α SIGNALING PATHWAY IN ERYTHROPHAGOCYTOSIS IN CANINE MACROPHAGE-LIKE CELLS

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Background: Immune mediated hemolytic anemia (IMHA) is devastating disease in dogs mediated by antierythrocytic antibodies and/or complement which target erythrocytes for macrophagic erythrophagocytosis. The role of altered phagocytosis inhibitory pathways has not been evaluated in IMHA. A major inhibitory pathway is activated when macrophage signal regulatory protein α (SIRP α) binds CD47, the “don’t eat me” signal on host cells.

Objective: Our first objective was to develop an in vitro method to investigate erythrophagocytosis using canine macrophage-like cells (DH82). Our second objective was to determine whether CD47 – SIRP α signaling regulates erythrophagocytosis in DH82 cells.

Methods: DH82 cells were incubated with or without normal canine erythrocytes labeled with carboxyfluorescein succinimidyl ester (CFSE), and uptake of erythrocytes was analyzed by flow cytometry. CD47 expression on canine erythrocytes was evaluated by flow cytometry, whereas SIRP α mRNA expression in DH82 was shown by PCR. We transfected DH82 cells with SIRP α siRNA targeting sequences and measured knockdown efficiency with quantitative PCR (qPCR).

Results: Erythrophagocytosis was detected in approximately 3% of DH82 cells. Canine erythrocytes express surface CD47 protein, and DH82 cells express SIRP α mRNA. Transfection with siRNA targeting SIRP α sequences resulted in an approximately 6-fold reduction in SIRP α mRNA expression.

Conclusion: Canine macrophage-like DH82 cells display minimal erythrophagocytosis of normal canine erythrocytes. Canine cells express CD47 protein and SIRP α mRNA. These tools provide groundwork for identifying potential therapeutic targets to modulate IMHA in veterinary patients.

86: TRIGLYCERIDE-RICH ASCITES AND PANCREATITIS IN COMPANION ANIMALS: A CASE SERIES

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Background: Pancreatitis is a highly morbid and fatal disease amongst companion animals, however a standard diagnostic protocol has not yet been established. Triglyceride-rich ascites (TRA) associated with pancreatitis have been scarcely reported in people, but have not yet been reported in domestic animals. The underlying pathophysiology of this association is poorly understood.

Objective: Describe 5 cases of pancreatitis-associated TRA in dogs and cats.

Methods: Patients with concurrent imaging, laboratory, and clinical evidence of pancreatitis and ascites were prospectively selected.

Results: Clinical signs included hyporexia, lethargy, and abdominal distension and pain. Laboratory analysis characterized abdominal effusions as pure or modified transudates (total protein = 0.7 – 4.2 g/dL; nucleated cell count = 60 – 2020 cells/mL). Cell predominance was variable, but all samples showed Sudan III positive staining. Triglycerides varied between 171 – 418 mg/dL (ratio to serum = 1.2 – 4.8), and cholesterol between 13 – 97 mg/dL. Additional confirmed diagnoses included high-grade gastrointestinal lymphoma (n = 2), chronic kidney disease (n = 2), right-sided congestive heart failure (CHF, n = 2), protein-losing enteropathy (PLE, n = 1), and fluid overload (n = 1).

Conclusions: It is possible that gastrointestinal lymphoma and PLE were associated with lymphangiectasia and an effusive chylous component could be extrapolated. CHF is known to cause chylothorax, but not chylous ascites. Detection of lipid in abdominal fluid through special staining and biochemical analysis might improve the diagnostic sensitivity of pancreatitis. Further studies investigating the systemic pathophysiology and potential prognostic association between TRA and pancreatitis are warranted.

87: DEVELOPMENT OF A DEEP-LEARNING ARTIFICIAL INTELLIGENCE DIAGNOSTIC SUPPORT TOOL FOR SPECIES AND TISSUE AGNOSTIC IDENTIFICATION OF MITOTIC FIGURES IN DIGITAL PATHOLOGY STUDIES

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The potential utility of digital pathology has become a subject of significant interest for both diagnostic and research pathology institutions. The Charles River Laboratories-Deciphex partnership develops digital and deep learning-enabled artificial intelligence (AI) tools that provide decision support for pathologists' evaluations of general

toxicology studies. These tools are usually developed using segmentation or object detection approaches. For rare or individual cell diagnostics such as done with mitoses, an object detection approach has potential advantages in terms of sensitivity and precision. We began work to develop a decision support tool using deep-learning AI digital pathology classifiers for identifying and quantifying mitotic figures across tissue types and species. A pilot object detection classifier was developed in the rat liver. This classifier was then applied to select rat kidney slides and performance was evaluated qualitatively by the trainer with a microscopic hematoxylin and eosin-based ground truth using the literature and quantitatively using F1 scores (harmonic mean of precision and accuracy). Classifier improvements were accomplished using corrective annotations (negative mining) and including additional training slides. Preliminary results indicate that a cross-tissue mitotic figure object identification and quantification classifier using deep learning artificial intelligence may be an effective diagnostic support tool in digital pathology.

88: USE OF DEEP LEARNING ARTIFICIAL INTELLIGENCE (AI) ALGORITHMS FOR QUANTIFICATION OF MICROSCOPIC ANATOMIC STRUCTURES AND ENDOLITHIC FUNGUS IN ACROPORA SPP. CORALS

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Nursery cultured stony coral fragments of *Acropora spp.* were maintained in small reef aquarium and exhibited gross lesions of “Slow Tissue Necrosis”, including irregular basal plate growth, overgrowth of surface algae, decreased polyp extension and decreased fluorescence. When cut on sagittal plane, affected corals revealed unorganized calyx segmentation and coalescing pillars of skeleton (aragonite). Fragments were fixed in 10% buffered formalin, sectioned with a diamond saw, decalcified with calcium EDTA, routinely processed and stained with PAS fungal and H&E. Histopathology revealed endolithic fungal overgrowth penetrating the calicodermis, excessive aragonite deposition, calicoblast and desmocyte hypertrophy, zooxanthellae depletion and production of laminar coral acid-rich proteins (CARPs). Slides were digitalized, loaded into the HALO AI™ Software (Indica Labs) and subjected to deep learning artificial intelligence (AI) neural networks using multiple algorithms. Structures were annotated manually by computer cursor and placed into classification order of increasing hierarchy (all coral tissue < epidermis < gastrodermis < mesoglea < gastrodermal cavity < calicodermis < non-coral organisms < fungal hyphae). Each classifier program was trained over 50,000 iterations to achieve the lowest Cross-Entropy level, indicative of the AI accurately interpreting the slide. Quantitative analysis compared grossly unaffected vs affected specimens, revealing that severe endolithic fungal overgrowth was correlated with greater deposition of aragonite skeleton, a decrease in gastrodermis area and decreased numbers of zooxanthellae. In summary, the use of deep learning AI, paired with traditional histopathology, allows classification and quantification of coral microanatomy and halobiont, making this a valuable tool for evaluating mass bleaching and chronic die-off events.

89: BLASTOMYCES HELICUS PNEUMONIA IN A DOMESTIC CAT IN COLORADO

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A 3-year-old male castrated domestic short hair cat presented for postmortem evaluation at Colorado State University Veterinary Diagnostic Lab after clinical decline associated with labored breathing, pyrexia, and a pulmonary mass identified radiographically. On gross examination, the lungs were found to have severe nodular to regionally extensive pneumonia. The nodules, approximately 100 in total, were raised, miliary to coalescing, irregularly circumscribed, and pale tan to white. The largest coalescing mass encompassed almost 95% of the right caudodorsal lobe. On histopathology, there were an abundance of foamy macrophages, multinucleated giant cells, and variably degenerate neutrophils effacing alveolar spaces and septa and occasionally dissecting into terminal bronchioles. Scattered throughout the inflammatory population are moderate numbers of intracytoplasmic and extracellular ovoid, non-staining, thin-walled, intracytoplasmic, narrow-budding yeast organisms. The tracheobronchial lymph node had severe, multifocal sinusoidal histiocytosis, and lymphofollicular hyperplasia. Fungal culture and sequencing of the lung revealed *Blastomyces helicus* and *percursus*. *B. helicus* is an emerging fungal organism that can cause fatal pulmonary and systemic disease in companion animals and immunocompromised patients. However, not much is known about its epidemiology or clinical presentation. A review of the literature found 10 human cases and 5 veterinary cases of *B. helicus* infection in western regions of Canada and the United States. This literature and coinciding diagnostic case could suggest the shifting westward geographic distribution of *Blastomyces* species from the traditional Ohio river valley that is commonly associated with *B. dermatitidis*.

90: COMPARISON OF METHODS TO ESTIMATE CANINE IGG4 CONCENTRATION IN SERUM

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Background: Accurate quantification of polyclonal IgG4 is useful in diagnosing and monitoring human IgG4-related disease (IgG4-RD) and other infectious processes. It may be helpful in dogs. Densitometric measurement from serum protein electrophoresis (SPE) may not accurately capture low concentration and polyclonal IgG4. Immunofixation allows only for a semiquantitative assessment of IgG4 quantity. Both commercial and lab developed IgG4 ELISA assays have been reported. These assays have not been compared.

Objective: Evaluate performance of methods for assessing canine serum IgG4.

Methods: A canine-specific serum IgG4 ELISA assay (ELISA_{LAB}) which used commercially available capture antibodies and canine monoclonal IgG4 as a standard was used to evaluate 111 archived canine serum samples that had also SPE and IgG4 immunofixation (IF). ELISA_{LAB} was compared with the IgG4 estimated from SPE

densitometry (Dens_{SPE}), a subjective IF IgG4 labeling grade (IF_{Grade}), and a commercial canine IgG4 ELISA kit (ELISA_{COM}).

Results: The ELISA_{LAB} assay had acceptable CV (11.3%, 95%CI=2.8-20.5%) and was deemed acceptable. There was moderate correlation between ELISA_{LAB} and DENS_{SPE} (Spearman's $\rho=0.80$, 95%CI=0.60-0.91) and IF_{Grade} (Spearman's $\rho=0.71$, 95%CI=0.60-0.80) though the data suggested they could not be used interchangeably based on Bland-Altman plot analysis. All samples were outside the linearity limits of the ELISA_{COM} assay, despite appropriate dilutions, suggesting ELISA_{COM} was not comparable with the other assays and that the ELISA_{COM} assay needed to be further evaluated.

Conclusion: Evaluation of the clinical utility of the ELISA_{LAB} assay is warranted to determine if it provides diagnostically and therapeutically relevant results.

91: A RAT HEPATOCELLULAR CARCINOMA MODEL FOR INVESTIGATING MOLECULAR PHENOTYPES ASSOCIATED WITH TUMOR STIFFNESS

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Background: Hepatocellular carcinoma accounts for 90% of liver cancers and progression is known to be modulated by the immune system. Tumor stromal heterogeneity has been shown to negatively impact immune surveillance in the tumor microenvironment. Few imaging techniques accurately predict stromal properties that correlate with prognostic and molecular features in liver cancer. This study aimed to determine how tumor stiffness correlates with immune cell infiltration and elucidate the molecular underpinnings associated with tumor stiffness using the non-invasive technique of shear wave elastography (SWE).

Methods: Rat hepatoma cells expressing green fluorescent protein were implanted in the liver of buffalo rats. Three weeks post implantation, tumor engraftment was verified by ultrasound and SWE data was collected. Tumors were then collected, processed, and stained using a Leica Bond Rx. Two multiplex immunofluorescence panels were utilized to compare soft and stiff tumors and analysis was performed with HALO software. GraphPad Prism v.9 was used to analyze data with a p value less than 0.05 considered significant.

Results: SWE showed variable tumor stiffness ranging from 2.0 to 3.5m/s with a mean value of 2.7m/s. Vimentin+ mesenchymal cells and GFAP+ hepatic stellate cells were significantly increased in the tumor liver interface (TLI) when compared to the tumor center.

Conclusions: Findings suggest that shear wave elastography may be useful as a non-invasive technique for predicting molecular phenotypes. CD45+ immune cells were predominantly limited to peripheral areas at the liver- tumor interface.

92: WE'VE GOT A BLEEDER: RUPTURE OF ADRENAL CORTICAL CARCINOMA LEADING TO ACUTE RETROPERITONEAL HEMORRHAGE IN A DOG

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A 12-year old, spayed female poodle presented with progressive acute weakness over a 24- hour period. She was non-ambulatory, tachycardic, and exhibited mild pain on deep abdominal palpation. Hematocrit was 33% at presentation. Radiograph findings revealed a retroperitoneal mass effect displacing abdominal viscera, including the descending colon and kidneys, ventrally. Due to poor prognosis, she was humanely euthanized. Gross necropsy findings included marked acute retroperitoneal hemorrhage extending to the diaphragm. The right adrenal gland was expanded along the medial aspect by a ruptured, large, thin-walled mass with poor demarcation and large amounts of fibrin and coagulated blood. Approximately 50% of the normal adrenal tissue remained intact, and the mass remained continuous with the adrenal cortical parenchyma. There was no evidence of vascular invasion to the caudal vena cava or right renal artery. The left adrenal gland appeared grossly normal. Histopathologically, the right adrenal gland was expanded by a highly cellular neoplasm admixed with marked hemorrhage, consistent with an adrenal cortical carcinoma. Cortical carcinomas are most frequently diagnosed in dogs and are usually functional, leading to hyperadrenocorticism; however, this patient did not display classic clinical signs of Cushing's disease, such as polyuria and polydipsia, and the retroperitoneal hemorrhage was likely the first clinical sign of an adrenal tumor. Furthermore, adrenal cortical tumor rupture resulting in retroperitoneal hemorrhage in dogs is rare, and few case studies have been published. However, acute retroperitoneal hemorrhage secondary to adrenal cortical rupture has been well-documented in humans and is often congruent with a worse prognosis.

