SP-1: DEVELOPING AND OPTIMIZING A FLOW CYTOMETRY-BASED ASSAY FOR FELINE INFECTIOUS PERITONITIS VIRUS

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Feline Infectious Peritonitis (FIP) is a deadly viral disease affecting cats that are carriers of the almost ubiquitous feline enteric coronavirus (FECV). Infection with FECV is mostly asymptomatic and transient, but it can mutate into FIP virus (FIPV) causing non-specific, and ultimately fatal clinical signs. Diagnostic testing for FIP is often ambiguous and usually presumptive, with most available tests unable to distinguish between the two different pathotypes (FECV vs. FIPV). A definitive diagnosis is usually made by biopsy and examination of tissue post-mortem. The aim of this study is to develop and optimize a flow cytometric assay for the detection of FIPV within fluid macrophages. Felis catus whole fetus (fcwf) cells are grown in standard media. Cells are infected with FIPV, along with sham-infected controls. Then, cells are harvested, fixed, permeabilized, and stained with anti-vimentin and anti-feline coronavirus antibodies and analyzed by flow cytometry. Preliminary results show that flow cytometry detects stained FIPV within fcwf cells, and eventually, we expect this within fluid macrophages from feline patients suspected of having FIP. We anticipate that our flow cytometry-based assay for FIP will be more diagnostically reliable (higher specificity) than currently available assays. A definitive antemortem diagnosis is crucial to veterinarians and clients when it comes to making treatment or euthanasia decisions surrounding FIP. The next steps for this study include testing fluid samples from suspected FIP patients in a clinical diagnostic trial.

SP-2: METASTATIC HEPATOCELLULAR CARCINOMA IN AN AYE-AYE

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Aye-aye (*Daubentonia madagascariensis*) is the largest nocturnal primate in the world and the only extant species of the genus *Daubentonia.* Aye-aye is considered “endangered” on the IUCN Red List of Threatened Species. Spontaneous neoplasms in aye-ayes are extremely rare with a total of only three cases reported prior to this date: a cerebral glioblastoma, a cholangiocarcinoma, and an oral squamous cell carcinoma. A 19-year-old, female aye-aye was euthanized after a five-day history of severe respiratory distress. Formalin-fixed paraffin-embedded tissues were submitted for microscopic examination. The hepatic parenchyma was replaced by densely cellular neoplastic hepatocytes arranged in solid sheets and irregular, multilayered trabeculae lacking normal lobular architecture. The mass contained multiple dilated, tortuous vascular channels. Neoplastic hepatocytes were polygonal with discrete cell borders, moderate amounts of eosinophilic cytoplasm containing occasional microvesicles, round and central nuclei with coarsely stippled chromatin with often one prominent nucleolus. Anisocytosis and anisokaryosis were moderate with scattered binucleated cells. Mitoses were rarely observed in 2.37 mm^2. There were lobules of well-differentiated adipocytes, necrosis, hemorrhage, aggregates of hemosiderophages, and small amounts of fibrin deposits. Similar neoplastic cells were observed in the lungs and stomach. Intravascular

neoplastic thromboemboli were noted in the heart and kidneys. Hepatocellular carcinomas are more common than adenomas in prosimians and have more pronounced cellular pleomorphism and anisocytosis than described in other mammals. Even though spontaneous hepatocellular carcinomas are considered to be a relatively common neoplasm in prosimians, to the authors' knowledge, this is the first case of metastatic hepatocellular carcinoma in a captive aye-aye.

SP-3: CANINE ACTINOMYCES PERICARDITIS

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Background: A 20-month-old, intact female German Shepherd presented deceased with a history of progressive weight loss and anorexia. The dog was found dead overnight. **Objective:** Histopathology, histochemistry, bacteriological culture, MALDI-TOF MS, and whole-genome sequence was performed to confirm the etiological diagnosis on an epicardial mass. **Results:** At necropsy, the thoracic cavity contained approximately 1 liter of serosanguineous fluid with flocculent debris. Moderate atelectasis of the lungs was present. The pericardial sac was markedly enlarged and filled the ventral mediastinum and thoracic inlet. The consistency was friable, with numerous firm coalescing nodular foci. On cut section, the nodules have necrohemorrhagic centers. No pericardial adhesions were observed. Histologically the epicardium was replaced by large, multifocal to coalescing areas of pyogranulomatous inflammation, areas of necrosis, and by abundant mature granulation tissue. Necrotic centers have large aggregates of Gram + and GMS + filamentous bacteria intermixed with abundant finely granular basophilic to eosinophilic material radiating from the necrotic centers (Splendore-Hoeppli material; "sulfur granules"). The presence of *Actinomyces bowdenii* was confirmed by routine culture and MALDI-TOF MS. Additionally, *Klebsiella pneumoniae* was isolated from pericardium and thoracic fluid. **Conclusions:** *Actinomyces bowdenii* is a normal commensal of the oral mucosa. Penetrating wounds of the oropharyngeal and esophageal mucosa predispose to its dissemination. However, dogs that develop pyogranulomatous pleuritis/pneumonia are commonly found in association with inhalation of grass seeds or parts of forage, confirmed by the presence of foreign body–like lesions. In this case, the lung and pleura were histologically unremarkable, suggesting a potential oropharyngeal origin.

SP-4: MESSENGER RNA TREATMENT TO INDUCE EXPRESSION OF BOVINE CATHELICIDINS TO COMBAT RESPIRATORY DISEASE PATHOGENS

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USDA surveys indicate that bovine respiratory disease (BRD) is the leading cause of mortality in U.S. feedlot cattle with a financial impact in excess of $1 billion annually. BRD results from a combination of management-related stressors, naïve host immunity, and exposure to viral pathogens such as bovine respiratory syncytial virus (BRSV) and

bovine coronavirus (BCoV), as well as bacterial pathogens such as *Pasteurella multocida*. Various methods to control BRD have either been inadequately effective or have led to antimicrobial resistance. A novel prevention approach is to induce expression of innate antimicrobial molecules, cathelicidins, on the respiratory surface to induce rapid protection against BRD. Cathelicidins are a group of innate immune mediators produced by neutrophils and epithelial cells with direct antimicrobial, inflammatory, and chemotactic effects. Our overall objective is to transfect bovine cells with synthetic mRNA encoding for bovine cathelicidin 2 (Bac5) and cathelicidin 5 (BMAP28) and to measure the antibacterial and antiviral effects of expressed peptides against the *P. multocida* and bovine coronavirus (BCoV). The current methodology is to transfect bovine kidney cells with synthetic mRNA and then test the supernatant and cell lysate of the transfected tissue culture against *P. multocida* and BCoV. The current research shows peak expression at 24 hours utilizing a reporter sequence, NanoLuciferase. This is preliminary research to serve as a foundation for further *in vitro* trials, then *in vivo* trials once *in vitro* antimicrobial efficacy is confirmed.

SP-5: CORONARY ARTERY FIBROMUSCULAR DYSPLASIA AS A CAUSE OF MYOCARDIAL INFARCTION AND SUDDEN DEATH IN A PUPPY

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A 4-month-old, male Siberian husky died suddenly 1 day after being obtained from the breeder. At postmortem examination the lungs failed to collapse (edema), had a moderate amount of pericardial effusion, mild to moderate cardiomegaly, and multifocal to coalescing white to gray streaks and areas of discoloration affecting the endocardium and myocardium of the left ventricular papillary muscles, occasionally bordered by hemorrhage. Histologically the myocardium had increased number and size of coronary vascular profiles that were tortuous, had irregular walls characterized by irregular proliferation and fibrosis of the tunica intima, media, and adventitia, as well as occasional reorganizing thrombi in the lumen. Associated with these abnormal vessels there were areas of myocardial degeneration, necrosis, and mineralization consistent with subacute myocardial infarctions. Based on these findings a diagnosis of coronary artery fibromuscular dysplasia with secondary myocardial infarction was considered the cause of death. Fibromuscular dysplasia is a well-characterized noninflammatory vasculopathy in humans that has rarely been reported in veterinary species. This report details the vascular lesions in a case of sudden death in a puppy with fibromuscular dysplasia of the coronary arteries.

SP-6: AVIPOXVIRUS-ASSOCIATED PROLIFERATIVE AND NECROTIZING DERMATITIS IN TWO HOUSE SPARROWS (PASSER DOMETICUS)

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A male and a female house sparrow (Passer domesticus) respectively caught in separate rural or urban areas of mid-Michigan were euthanized due to inability to perch

or fly, alopecia, and yellow exudative skin sores or growths. On necropsy, there were multifocal to coalescing raised yellow nodules over the feathered and glabrous portions of skin of both birds. Such regions histologically corresponded to well-demarcated, plaque-like or exuberant exophytic protrusions from the skin surface due to hyperplasia of the epidermis and follicular epithelium with necrosis of apical portions of the expanded epithelium. Viable hyperplastic epithelial cells had intracellular edema and cells throughout the hyperplastic stratum spinosum and within regions of superficial necrosis contained 20-30 µm in diameter, hypereosinophilic intracytoplasmic inclusions consistent with Bollinger bodies. PCR for poxvirus 4b core protein on portions of affected skin yielded an approximately 570 bp amplicon that had 100 percent sequence homology to an avipoxvirus stain previously reported in a sparrow. Avipoxvirus, known colloquially as bird pox or avian pox, is a classic infectious disease in birds. There are many strains of avipoxvirus, and most are species-specific. The disease comes in two forms, cutaneous and diphtheritic. Mortality associated with the cutaneous form of bird pox is often mediated by secondary issues such as emaciation due to the impendance of flight and feeding. Outbreaks of avian pox in wild species can lead to high morbidity and mortality in affected populations.

SP-7: ENTOMOPATHOGENIC METARHIZIUM SP. FUNGAL RHINITIS IN A DONKEY

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A 21-year-old donkey (*Equus asinus)* jenny presented for a 9-week history of chronic, progressively worsening bilateral mucopurulent nasal discharge and depressed respiratory effort. Radiographs showed bilaterally increased opacity of the nasal passages. Endoscopy revealed multiple masses bilaterally in the nasal passages with the left side more affected than the right, potentially deviating the nasal septum to the right. Histopathology of a nasal mass showed severe, diffuse, chronic eosinophilic and granulomatous rhinitis with numerous intra-lesional hyphae. The hyphae were GMS positive, 10-15 µm in diameter, variably septate, with non-parallel walls, dichotomous branching, and occasional bulbous swellings. Panfungal PCR of the ITS and LSU regions yielded 100% and 99.67% matching identity to *Metarhizium* sp*.*, respectively. These results, in conjunction with comparisons of fungal morphology, confirmed *Metarhizium* sp*.* as the causative agent in this case. *Metarhizium* is an entomopathogen of insects commonly used as a pesticide. *Metarhizium* infection has been confirmed in a few rare human cases and in one case report of a cat presenting with eosinophilic and granulomatous rhinitis with similar histologic morphology. This is the first reported case of infection with *Metarhizium* sp. in an equid.

SP-8: DOES BLUEBERRY POLLEN INCREASE THE SUSCEPTIBILITY OF HONEY BEES (APIS MELLIFERA) TO EUROPEAN FOULBROOD DISEASE?

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European foulbrood (EFB) is a stress-induced disease of honey bee brood (*Apis mellifera*) caused by the bacterium *Melissococcus plutonius* which occupies the larval midgut and competes with larvae for ingested food. EFB has become increasingly observed in colonies that subsist on blueberry pollination. This increased EFB incidence is thought to be due to blueberry pollen's low nutritional value. The objective of this study was to determine if pollen collected by bees involved in blueberry pollination makes honey bee larvae more susceptible to EFB infection using a previously established EFB in-vivo infection model. Genetically related honey bee larvae were exposed to control treatments or to low or high doses of *M. plutonius* in 2 µl of aqueous solution. The inoculated larvae were raised in paired nucleus colonies supplemented with either blueberry or multifloral pollen (collected from Saskatchewan prairie). Larval survival and pollen consumption were measured six days post-infection. Colonies that received blueberry pollen experienced 16-26% and 18-23% higher larval survival than those that received multifloral pollen in the low and high EFB dose treatment groups, respectively ($p < 0.005$). The amount of pollen consumed was random however, colonies that consumed less pollen had lower larval survival in response to EFB infection. This study suggests that blueberry pollen does not increase the susceptibility of bees to EFB infection. Interestingly, our results also show that larval survival may partly depend on a colony's total pollen consumption, regardless of pollen type provided, which confirms the current understanding of EFB as a disease of nutritional stress.

SP-9: LEIOMYOSARCOMA IN THE RIGHT-WING OF A BUDGERIGAR (MELOPSITTACUS UNDULATUS)

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Formalin fixed tissues from an adult budgerigar were submitted for histopathology with the main tissue of interest being a large right-wing mass. Prior cytologic evaluation of the mass revealed significant numbers of neoplastic mesenchymal cells. Histopathology of the right-wing mass was composed of densely cellular streams and swirls of poorly demarcated, neoplastic mesenchymal cells. Immunohistochemical staining of the neoplastic population were positive for smooth muscle actin and muscle specific actin but negative for vimentin. Based on the histologic characteristics and immunohistochemical staining, the right-wing mass was diagnosed as a leiomyosarcoma. Leiomyosarcoma is mesenchymal neoplasm originating from smooth muscle tissue present in the gastrointestinal tract, urogenital tract, or cutaneous tissues that have been documented in few animal species. Within avian species, psittacines represent a small cohort of leiomyosarcoma cases that are typically isolated within the coelomic cavity, most commonly the splenic trabeculae, and may metastasize to

visceral organs. Though uncommon, isolated cases of cutaneous leiomyosarcomas have been documented in Psittaciformes, Galliformes, and Columbiformes. These tumors were located in the tissue covering the coelomic cavity and the tissue of the inner wing with the tumors possibly originating from the vascular smooth muscle and/or the feather follicle smooth muscle.

SP-10: MYCOPLASMA BOVIS MENINGITIS VIA VESTIBULOCOCHLEAR NERVE IN A HEIFER

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A 3-month-old female black Angus calf presented for neurological signs including ataxia and opisthotonos. On gross examination, both tympanic bulla were occluded by opaque, soft to crumbly, tan-white material, and the underlying mucoperiosteum was diffusely reddened. Coalescing pale nodules thickened the periosteum of the cranial vault around the internal acoustic meatus. The cranioventral lungs had bronchopneumonia with caseonecrotic nodules. Histopathology revealed inflammation with foci of caseous necrosis in the vestibulocochlear nerve, lymphoplasmacytic and histiocytic encephalitis with meningitis in the brainstem, and caseonecrotic bronchopneumonia in the lung. Multiple bacteria including *Mycoplasma bovis* were isolated from lung and middle ear. The gross, histopathology and microbiology findings indicated *M. bovis* as the primary cause for pneumonia, otitis and meningitis in the calf. Whereas hematogenous infection can be considered for *M. bovis* infection of joints and meninges, in this case the caseonecrotic auditory neuritis and meningitis indicate spread of *M. bovis* from the middle ear to the brain via the vestibulocochlear nerve. The cause of this neurological condition in calves has been poorly understood, but this case provides evidence for *M. bovis* as a cause, with invasion from the middle ear via the vestibulocochlear nerve as a route of entry to the brainstem.

SP-11: EFFECT OF SALINE REPLACEMENT ON HEMOGLOBIN AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATIONS IN LIPEMIC CANINE BLOOD SAMPLES

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Background: Effects of lipemia on hematological analysis is a common problem in veterinary diagnostic laboratories. Lipemia causes interference in spectral absorption assays, leading to erroneous overestimation of hemoglobin concentration and calculation of mean corpuscular hemoglobin concentration (MCHC). Saline replacement of lipemic plasma to avoid interference is commony performed in human diagnostic laboratories but has not been evaluated in canine samples. **Objective:** To determine if replacing lipemic plasma with saline significantly decreases hemoglobin and MCHC concentrations. **Methods:** Lipemic K_3-EDTA canine blood samples were obtained or were artificially created by adding a small volume of lipemic serum. Initial data was obtained using a Sysmex XT-2000iV analyzer. Samples had lipemic supernatant removed and replaced with the same volume of 0.9 % saline; data was obtained again.

Data were compared via 1-Factor ANOVA analysis with significance at $p<0.05$. **Results:** Saline replacement significantly reduced hemoglobin concentrations ($p<0.01$) by 83% for both natural and artificial samples. MCHC reduced by 88% ($p=0.01$) for natural and 95% ($p<0.01$) for artificial samples when compared to initial lipemic samples. There were no significant differences in data between natural and artificial samples. **Conclusions:** The significant reduction in hemoglobin and MCHC concentrations following saline replacement supports that this technique removes interfering effects of lipemia on Sysmex XT-2000iV data obtained from K_3-EDTA canine blood samples. As anemia classification depends on MCHC, this procedure should translate into more accurate evaluation of canine patients. Assuming similar results with other analyzers, adopting it as a standard operating procedure should be considered by veterinary diagnostic laboratories.

SP-12: MULTIFOCAL FUNGAL OSTEOMYELITIS CAUSED BY TALAROMYCES SPP IN A DOG

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A nine year old, female spayed Great Dane presented to the Texas A&M Veterinary Medical Teaching Hospital with a history of chronic neck and shoulder pain and progressive hindlimb ataxia. MRI of the cervical spinal cord revealed multiple aggressive bone lesions of the cervical vertebrae and right scapula, with lymphadenopathy of the right axillary, right superficial cervical, sternal, and cranial mediastinal lymph nodes. Thoracic radiographs were consistent with MRI findings. Ultrasound guided fine needle aspirates of lymph nodes and a right scapular mass were prepared with modified Wright's stain. Right superficial cervical lymph node cytologic findings revealed a mildly reactive lymph node with moderate macrophagic inflammation and poorly staining fungal hyphae. The fungal hyphae were septated, 5-6 um in diameter with varied lengths and a thin 1-2 um non-staining wall, and some displayed branching. The right scapular mass contained marked macrophagic and mild neutrophilic inflammation and fungal hyphae morphologically similar to those found in the right superficial cervical lymph node. The cytologic findings were consistent with disseminated fungal infection. An *Aspergillus* antigen EIA for detection of *Aspergillus* galactomannan was negative. A panfungal PCR showed the presence of *Talaromyces* species closely related to *Talaromyces borbonicus*. Fungi of the *Talaromyces* genus have been reported to cause granulomatous arthritis and focal osteomyelitis in two dogs, but this is the first case to demonstrate evidence of a severe multifocal presentation. This patient was not known to be immunocompromised prior to clinical signs.

SP-13: A case report of Cryptosporidium-like protozoal infections within the epithelial lining of the small intestine of Micropterus salmoides.

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Cryptosporidium is a zoonotic pathogen endemic to a variety of wild and domesticated animals. The prevalence and histologic pathogenesis of Cryptosporidium in aquatic species is poorly understood, as is their impact on public health. The demand for farm-raised fish increases every year along with the risk of fish-borne diseases. Cryptosporidium is a gregarine species and is expected to appear on the apical side of the digestive tract on histologic evaluation, but there are previous reports showing an alternative histological appearance of Cryptosporidium infection in aquatic species. A case study of farm-raised largemouth bass (*Micropterus salmoides*) with symptoms of enteritis was evaluated histologically for the presence of enteric pathology. As largemouth bass are both a game fish as well as a farmed fish, the prevalence of Cryptosporidium would pose an inherent risk to consumers who eat improperly prepared fish. Hematoxylin-eosin, periodic acid-Schiff, and Ziehl-Neelsen stained sections of the small intestine showed 5µm encapsulated spherules within and adhered to the epithelial lining that were consistent with both the traditional and alternative induced Cryptosporidium infection in aquatic species. Additionally, sections of the small intestine were retrieved from other largemouth bass part of the case study. The protozoa in question were recovered using immunomagnetic separation and visualized using direct immunofluorescent anti-cryptosporidium monoclonal antibodies. The direct immunofluorescence developed for *C. parvum* was weakly positive for the protozoa in question, indicating either some cross-reactivity of species or a more novel type of pathogen. Further characterization of these Cryptosporidium-like protozoa could have significant implications on public health.

SP-14: CREATION AND VALIDATION OF MYCOBACTERIUM TUBERCULOSIS GRANULOMA RECOGNITION SOFTWARE

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Acid Fast stained bacilli are a key diagnostic visual feature observed in the lung tissue of Diversity Outbred mice infected with *Mycobacterium tuberculosis.* Previous studies have exploited the artificial intelligence (AI) potentials to detect classic features of TB granuloma such as regions of neutrophil clusters or macrophage foci. However, the pathogenesis incites a complex host response beyond the well-recognized cells in *M. tuberculosis* infection. Currently, no published automated algorithms can detect individual, singular cells within TB granuloma, such as individual Acid Fast bacilli or individual nuclei. Here, we used whole slide lung images from *M. tuberculosis* infected Diversity Outbred mice to train, validate, and performance test an algorithm to automatically detect and classify individual mycobacteria and macrophage nuclei. The performance of the proposed machine-learning model was evaluated using two main outcome measures. In the first layer, *M. tuberculosis* infected lung tissue was detected following the elimination of the non-tissue background. In the second layer, correct detection of potential regions, and correct classification of these regions into single AFBs, clumps of AFBsA , and normal nuclei were evaluated. The proposed AI algorithm can successfully detect lung tissue, normal nuclei, and AFBs with final error rates of 4.76%,1.76% , and 7.02%, respectively. Overall, our results provide a method to

convert the obtained visual (image) data into measureable, quantitative information suitable for statistical analysis. Furthermore, statistical data of the number, location, and distribution of these individual cells within TB granuloma improves our understanding of the disease.

SP-15: EROSIVE ENTERITIS AND DEATH DUE TO EIMERIA MACUSANIENSIS INFECTION IN AN ALPACA

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This report describes a case of erosive enteritis and death due to *Eimeria macusaniensis* in a 6-month-old alpaca stud. The stud was found laterally recumbent, inappetent, dehydrated, and deceased. Gross post-mortem examination revealed the presence of 700ml of clear yellow fluid in the abdominal cavity. The lumen of the small intestine was distended by gas and green watery contents, and its wall was thickened and discolored dark red. There was also diffuse dark red-black discoloration of the liver. Histopathologic examination revealed marked expansion of the mucosa of the small intestine by myriad intraepithelial schizonts, microgamonts, and macrogamonts that were morphologically consistent with *E. macusaniensis;* mixed inflammatory cell infiltrates and mucosal erosion were also associated. Histopathology of the liver demonstrated hepatocellular necrosis, hemorrhage, and multifocal amyloidosis. Fecal microscopy also confirmed the presence of *E. macusaniensis.* Because there was no overt evidence of coinfection with other pathogenic organisms, the death of this animal was considered due to the marked *E. macusaniensis* burden and associated erosive enteritis. *E. macusaniensis* is extremely important in South American camelids as a cause of acute or sudden death and can cause disease on its own, or via erosion of the intestinal mucosa, allowing a portal of entry for intestinal opportunists and commensals.

SP-16: DO HIGHLY PATHOGENIC ISOLATES OF MELISSOCOCCUS PLUTONIUS EXPLAIN OUTBREAKS OF EUROPEAN FOULBROOD DISEASE DURING BLUEBERRY POLLINATION?

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European foulbrood (EFB) is a bacterial disease of honey bee (*Apis mellifera*) larvae, caused by *Melissococcus plutonius.* This organism invades the larval midgut and can outcompete the larva for food, causing larval death. Recently, beekeepers have reported an increased incidence of EFB in blueberry pollinating colonies. The purpose of this study was to investigate if highly virulent strains of *M. plutonius* are responsible

for outbreaks of EFB during blueberry pollination. Eight *M. plutonius isolates* were collected from outbreaks of EFB in North America. Each isolate was grown in liquid media and positively identified as *M. plutonius* by MALDI-TOF mass spectrometry. One-day-old larvae were infected with 100 colony forming units of an *M. plutonius* isolate originating from a blueberry pollinating (n = 4) or a non-blueberry pollinating (n = 4) honey bee colony. Larval survival was monitored daily. Survival was analyzed using a Kaplan-Meier estimator and a Mantel-Cox log-rank test. *M. plutonius* isolates collected from outbreaks of EFB in blueberry pollinating colonies were not significantly more pathogenic than isolates from non-blueberry pollinating honey bee colonies. Pathogenic *M. plutonius* isolates caused 72% decrease in larval survival compared to control ($p < 0.001$). Interestingly, we found one non-pathogenic isolate of *M. plutonius* originating from a non-blueberry pollinating colony in Saskatchewan. This pilot study suggests that increased *M. plutonius* pathogenicity doesn't explain the increased incidence of EFB in blueberry pollinating colonies. Further investigation into environmental and host factors (e.g. colony nutrition and strength), is necessary to mitigate the risk of EFB during blueberry pollination.

SP-17: MENINGOENCEPHALOMYELITIS OF UNDETERMINED ORIGIN IN A CAT

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An unknown-aged female spayed cat presented to the University of Illinois Veterinary Diagnostic Laboratory for postmortem examination with a history of chronic neuropathy including fecal and urinary incontinence, circling, and right-sided limb hyperreflexia and delayed proprioception. Gross examination found yellow to tan turbid nasal discharge from the eyes and nares. The spinal canal had focally extensive hemorrhage with the cranial segments more affected. The brain, especially the caudal portions, had numerous vessels with expanded Virchow-Robbin spaces containing lymphocytes, plasma cells, macrophages, and few neutrophils up to 8 cell layers thick. Rarefaction of the parenchyma near most affected vessels was present with mild infiltrate of plasma cells, gitter cells, and hemorrhage and cell debris. Portions of meninges were expanded 2 to 5 layers thick with lymphocytes, macrophages, and plasma cells. The spinal cord also contained a dense infiltrate of lymphocytes, plasma cells, macrophages, and rare fibrin in the arachnoid space and surrounding nerve roots. Antemortem toxoplasmosis and feline infectious peritonitis (FIP) testing was negative. Postmortem FIP and feline herpesvirus PCR testing was negative, and culture of the brain yielded no growth. While common in dogs, there are few reports of meningoencephalomyelitis of undetermined origin (MUO) with no evidence of infectious agents in cats. Whole genome analysis identified a feline endogenous retrovirus (ERV-DC8) in the brain, which may be a contributing factor of the chronic neuropathy.

SP-18: DETERMINING POSTMORTEM INTERVAL IN THE LABORATORY MOUSE

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Estimating time of death in the laboratory mouse (*Mus musculus*) is important in determining the viability of samples collected for research in mice that die prior to scheduled collection. There are currently not well-established guidelines for determining the postmortem interval in the laboratory mouse based on gross appearance, and there are few studies that have temporally assessed autolysis in mouse tissue. Here we identified and scored gross and histologic postmortem changes during various time points with three main objectives: 1) Assess gross changes over time to assist pathologists, lab animal veterinarians, and veterinary technicians in estimating time of death; 2) Compare changes over time at both room temperature and under refrigeration; and 3) Identify and score autolysis histologically over time in each organ system under both conditions. Mice were euthanized and collected at 0, 3, 6, and 15 hours and daily up to 6 days postmortem after storage at either at 4 degrees Celsius or at room temperature for the allotted amount of time. We show that there are minimal gross changes that occur in a mouse carcass under both conditions. Gross autolysis began within three hours at room temperature, and storage at 4 degrees delayed these changes to a comparative level at about 3 days postmortem. We also identified that the rate of autolysis is markedly tissue dependent, and storage at 4 degrees significantly delayed autolysis histologically. Our results provide a starting point in determining the postmortem interval and viability of tissue for histologic analysis in the laboratory mouse.

SP-20: ESTABLISHING SERUM BIOMARKERS OF MYCOBACTERIUM TUBERCULOSIS INFECTION IN ASIAN ELEPHANTS

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Mycobacterium tuberculosis (M.tb) infection is a threat to elephant health and conservation and human public health worldwide. Cases of tuberculosis (TB), the disease due to *M.tb* in those susceptible, have occurred on multiple continents and affected both wild and captive elephants. Humans are the natural host for *M.tb* and main source of transmission to elephants. Infection can then spread from elephants to other elephants, to other mammals, and possibly back to humans. The biomarkers CXCL1, MMP8, IL-10, IFN-γ, and TNF-α are known to be involved in immune responses to *M.tb* infection in elephants or other similarly affected species. We used commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits to determine whether these five biomarkers are significantly elevated in the serum of *M.tb* positive elephants compared to *M.tb* negative elephants. We tested 101 samples from six *M.tb* negative elephants and five *M.tb* positive elephants, none of which had known clinical signs of disease. Biomarker concentrations were below the limit of detection for the assay in 100/101 (99%) samples for CXCL1, 98/101 (97%) samples for MMP8, 85/101 (84%) samples for IL-10, 75/101 (74%) samples for IFN-γ, and 45/101 (45%) samples for TNF-α. Multiple *M.tb* positive elephants did not have detectable levels of any of the five biomarkers, suggesting that these biomarkers are not substantially elevated in elephants infected with *M.tb*. More sensitive assays are needed to more accurately

determine the concentrations of these biomarkers in elephant serum and better evaluate their utility in diagnosing *M.tb* infection in elephants.

SP-21: RELATIONSHIP BETWEEN PARITY AND REPRODUCTIVE DISEASE IN MANAGED AFRICAN PAINTED DOGS (LYCAON PICTUS)

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Background: The managed female African Painted Dog (APD) population has been found to have high prevalence of reproductive disease, including cystic endometrial hyperplasia (CEH), adenomyosis, and pyometra. These diseases can result in infertility and even death. It is important to have females that can reproduce in order to maintain genetic diversity and sustain the managed APD population. **Objective:** The purpose of this study was to identify potential risk factors (e.g., parity, age, contraceptive use), associated with different reproductive diseases in order to provide zoos with management recommendations for female APDs. **Methods:** After a female APD died or was ovariohysterectomized, tissues were fixed in 10% formalin and shipped to the Reproductive Health Surveillance Program (RHSP). Tissues were photographed, dissected, embedded, sectioned, and stained with hematoxylin & eosin. Microscopic evaluation identified reproductive lesions. Spearman's correlation coefficients were produced using GraphPad Prism®. **Results:** Parity was not significantly correlated with cystic endometrial hyperplasia, adenomyosis, or pyometra. Age was positively correlated with presence of CEH ($r=0.539$, $p<0.001$), and adenomyosis ($r=0.631$, $p<0.001$). Time since last parturition was positively correlated with presence of CEH ($r=0.696$, $p=0.002$), adenomyosis ($r=0.513$, $p=0.03$), and endometritis ($r=0.511$, $p=0.03$). **Conclusions:** Preliminary data suggests that cystic endometrial hyperplasia and adenomyosis are, at least in part, degenerative changes. Data suggests that parturition could decrease the prevalence of CEH, adenomyosis, and endometritis. Breeding female APD at a younger age and breeding them frequently may allow them to reproduce before CEH and adenomyosis develop.

SP-22: MALIGNANT ORAL MELANOMA LEADS TO INTRAOCULAR MELANOCYTIC METASTASIS IN CANINE PATIENT

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Background: A 12-year-old neutered male Labrador Retriever, with a history of an incompletely excised malignant oral melanoma, presented to the Texas A&M Ophthalmology service with a concern of vision loss. Upon examination bilateral fibrinous uveitis was noted along with secondary glaucoma in the patient's left eye. Over the next month, uveitis in the left eye continued to progress and hyphema developed. A palliative enucleation was performed on the patient's left eye and the globe was submitted for histopathology. **Objective:** Determine the cause and origin of progressive uveitis and hemorrhage in the enucleated eye. **Methods:** Histopathology of the removed globe and immunohistochemistry using an immunohistochemical stain for

melanin-A. **Results:** Histologic analysis showed a poorly differentiated metastatic mesenchymal neoplasm lining the posterior iris surface, anterior lens capsule, ciliary body surface, and peripheral ventral retina. Immunohistochemistry determined that approximately 80% of the neoplastic cells exhibited strong cytoplasmic positivity for melanin-A, therefore indicating melanocytic origin. **Conclusion:** Oral melanoma is known for its high metastatic propensity and is one of the most common oral malignancies encountered in canine patients. While primary melanocytic tumors are common ocular neoplasms, secondary ocular melanocytic metastasis as seen in this case have rarely been documented. By observing malignant melanoma patterns of metastasis, development of effective treatment and prevention of this neoplasm could be improved in the future.

SP-23: PROTECTIVE EFFICACY OF A YEAST BYPRODUCT DIET AGAINST APEC CHALLENGE IN POULTRY- NECROPSY ANALYSIS

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Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis in poultry. Characterized by systemic extraintestinal infections, colibacillosis results in significant morbidity, mortality and carcass condemnation at slaughter. Consequently, APEC causes considerable economic losses for the poultry industry worldwide. Live vaccines and probiotics are commonly used by producers to prevent bacterial infections; however, have limited efficacy. In this study, we tested whether a proprietary feed containing a specific yeast byproduct could enhance gut health and offer protection against challenge with a highly-pathogenic APEC O78 strain. In each of two identical trials lasting 21 days, 120 chickens were assigned to eight groups: four groups were fed a control diet and four groups were fed the test diet. On day 14 of the study, birds were challenged either intratracheally or orally, with Phosphate Buffered Saline (PBS) or APEC O78. On day 21, the birds were euthanized and necropsied. Swabs of heart blood and air sac and tissues of liver, spleen, lung and ceca were aseptically collected. Additionally, all birds were scored on necropsy for evidence of lesions consistent with colibacillosis using a standard rubric. APEC was detected in birds fed the control diet at a similar rate to those fed the supplemented feed suggesting that the yeast byproduct had little protective effect in reducing the rate of infection detected. However, intratracheally challenged birds exhibited greater systemic disease and higher lesion scores. This study therefore provides valuable insight into the use of feed additives and their potential protective efficacy in poultry health.

SP-24: SPONTANEOUS NEOPLASMS IN CAPTIVE MEMBERS OF THE FAMILY VIVERRIDAE: A RETROSPECTIVE STUDY

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The family Viverridae comprises a unique and diverse set of carnivorous mammals including several species of civets and genets as well as oyans and binturongs. Few

reports of Viverridae have been published and much remains to be learned of these elusive species. The aim of this study was to review spontaneous neoplasms in captive Viverridae species seen in the diagnostic caseload of the pathology department at North Carolina State University College of Veterinary Medicine between 2014 and 2020. Eight total animals were received including six binturong (*Arctictis binturong*) and two common genets (*Genetta genetta*). On postmortem examination six animals, 6/8 (75%) – four binturong and two common genets – had one or multiple types of tumors including those of hepatocellular (2/6), renal (3/6), mammary (1/6), pulmonary (1/6), thyroid (1/6) or mast cell tumor (MCT; 1/6) origin. Of these six animals, 3/6 (50%) had primary renal neoplasms. Immunohistochemistry was pursued for the renal neoplasms (PAX8 and CD10) and mast cell tumor (KIT). The renal neoplasm results for PAX8 and CD10 showed variable immunopositivity (slide review pending) and the mast cell tumor showed weak to moderate diffuse cytoplasmic staining for KIT. While neoplasms in Viverridae have been reported in larger, more broad studies on neoplasia in zoo animals, this case series serves as the first to utilize immunohistochemistry in Viverrids, providing a baseline for future studies. Based on our findings, spontaneous neoplasia could be more common than previously published with older age as a predisposing factor, however, further investigation is warranted.

SP-25: INHIBITION OF CANINE GLIOMA CELLS BY MAP4K4 KNOCKDOWN AND TREATMENT WITH THE METASTASIS INHIBITOR METARRESTIN

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Gliomas are the second most common primary brain tumor in dogs and show many similarities to pediatric gliomas. For high grade gliomas, survival time is relatively short due to the aggressive nature of the disease and the lack of effective treatments. This study aims to test the effects of two potential anticancer compounds that have shown promising results in human cancer cells on canine glioma cells. Expression of MAP4K4, a serine/threonine kinase upregulated in many types of cancer cells and indicative of poor prognosis for survival, was targeted using specific siRNAs (200nM). Incubation for 48h resulted in high rates of siRNA transfection and led to significantly decreased MAP4K4 protein expression ($p<0.05$) as determined using a specific MAP4K4 antibody. Notably, live cell imaging revealed that downregulation of MAP4K4 results in decreased cell migration as indicated by a significant delay in wound healing of 5µm/15mins ($p<0.05$) compared to untreated control cultures at 6d post transfection. Cells were also treated with novel, first in class inhibitor metarrestin, which disrupts the perinucleolar compartment, a subnuclear body that is selectively formed in highly metastatic cancer cells. Cultures treated with 5µM metarrestin for 24 hours showed significantly less growth ($p<0.01$) compared to control glioma cells, suggesting an effect of this novel compound on glioma cells of canine origin. Our results suggest the potential applicability of both strategies for targeting canine glioma cells. Further analyses are underway to establish whether a two-pronged approach, using siMAP4K4 and metarrestin in combination, can further inhibit glioma cell proliferation and migration.

SP-26: TRACHEAL OSTEOCHONDROMA IN A FOUR MONTH OLD GOLDEN RETRIEVER PUPPY

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A 4-month-old female Golden Retriever presented for a 2-week history of wheezing. Cervicothoracic CT imaging revealed a smoothly marginated, variably mineral opaque, intraluminal tracheal mass at the level of C6. Tracheoscopy revealed an obstructive intraluminal mass covered by tracheal mucosa which obstructed 90% of the tracheal luminal diameter. The mass was removed by tracheal ring resection and anastomosis and submitted for histopathological examination. The tracheal submucosa was expanded by a wide-based, nodular mass composed of a thick outer layer of hyaline cartilage with endochondral ossification forming woven bone. There was a central, mature hematopoietic medullary cavity with erythroid, myeloid, and megakaryocytic precursor cells. The mass was diagnosed as a tracheal osteochondroma. Osteochondromas are benign osseous tumors formed by endochondral ossification of hyaline cartilage surrounding a mature hematopoietic medullary cavity. These tumors are rare but well-reported in the literature of young dogs (3-11 months old) with a predisposition for Husky and Malamute breeds. Masses can be solitary (monostotic osteochondroma) or multifocal (polyostotic osteochondroma or multiple cartilaginous exostoses) and increase in size at a rate proportional to the rate of patient maturation. These tumors cause pathology due to anatomical dysfunction of the appendicular skeleton or through a space-occupying mass effect. Surgical resection of lesions is typically curative. Non-tracheal osteochondromas have been reported to undergo malignant transformation into osteosarcoma, and although not reported, it is suspected tracheal osteochondromas may also have the potential to do so.

SP-27: RHODOCOCCUS EQUI INFECTION IN A GOAT

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A 5-month-old Nigerian Dwarf goat had a 5-day history of inappetence and cough. Bloodwork revealed high liver and renal enzymes. Radiographs and ultrasound revealed abundant abdominal and thoracic effusion and hepatic and pulmonary nodules. Due to poor prognosis, humane euthanasia was elected, and the goat was submitted for necropsy. Gross examination revealed a large volume of yellow, cloudy fluid in the thoracic and abdominal cavities. Throughout the liver, lungs and mesenteric lymph nodes were numerous variably-sized nodules. On cut surface, the nodules contained white-tan concentric rings. Histologically, the nodules in the liver, lungs, and lymph nodes were characterized by multifocal pyogranulomas with a center of gram-positive coccobacilli bacteria. A heavy growth of Rhodococcus equi was isolated via aerobic culture from the liver and lung. Gross and histologic lesions coupled with culture results are diagnostic for disseminated Rhodococcus equi infection. Rhodococcus equi is an aerobic, gram-positive, obligate, facultative intracellular coccobacillus which is most

commonly reported in foals, but can also affect goats, sheep, cattle, swine, dogs, cats, camelids, birds, wildlife, and humans. Soil and feces are natural reservoirs for R. equi, and infection can occur through ingestion or inhalation of the bacterium. Predisposing factors such as stress, illness, or immunosuppression likely play a role, especially in goats as they are typically resistant to infection.

SP-28: RETROSPECTIVE STUDY OF FISH CANCER PREVALENCE AND THERAPIES

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This study evaluated fish cancer through medical records from zoos, aquariums and exotic animal veterinarians. The parameters evaluated included geographic location, habitat type, signalment, location of cancer, type of cancer, survival time, and treatments provided. This data was entered into the Exotic Species Cancer Research Alliance (ESCRA) database, then the resulting statistics were compiled and analyzed. Out of 511 cases, sex was known from 28.4% of patients, and most were female (14.5%). A majority of the submitted animals were from a zoological park or aquarium (57.3%), followed by private ownership (9.8%). The most common species reported was koi (spp. Cyprinus rubrofuscus) (25.0%). The most commonly specified location of primary tumors was the neck (7.0%), with soft tissue being the most commonly affected body system (22.1%). Spindle cell sarcoma was the most common primary histopathology tumor diagnosis (12.7%). Most of the reported cancers were malignant (65.2%). Only five cases reported any form of treatment for the tumors, with surgery being the only therapeutic method used. Four of these patients had their cancer completely excised and one had incomplete excision. None of these animals were reported to have any complications or had died as a result of their surgical procedures. This data suggests that there is a deficit in documented clinical therapy of cancer in fish species. However, despite this limited data, surgical excision of cancer in fish may be beneficial for clinicians to strongly consider or further investigate when feasible for the patient and client.

SP-29: Autonomic Dysfunction with Features of Hereditary Dysautonomia in a Puppy

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A 10-week-old mixed breed female intact puppy with a clinical history of a few days of hemorrhagic diarrhea, lethargy, and anorexia died on the way to the RUVC clinic in St. Kitts. Death was initially attributed to dehydration, anemia, and subsequent shock. The postmortem examination revealed severe dehydration, red-tinged fluid fecal content, and moderately enlarged lungs with an area on the right cranial lobe being firm and hemorrhagic. Microscopically, main lesions involved the submucosal and myenteric plexus of the intestines with neurons showing loss of Nissl substance, some shrunken eosinophilic neurons, chromatolysis, peripheralized nuclei, decreased density of

neurons, vacuolization with satellite cell proliferation and low numbers of infiltrating lymphocytes. The lungs had a neutrophilic and histiocytic interstitial pneumonia with coccobacilli, compatible with aspiration pneumonia. Tissues submitted tested negative for canine distemper virus through PCR testing, and there were no lesions found to be consistent with canine parvovirus. In the brain, lesions known to be consistent with canine dysautonomia were not confirmed. Due to the lack of autolytic changes within the intestinal tissue, the extensive degenerative changes within the enteric plexus were consistent with those seen in cases of canine hereditary dysautonomia, which was not previously reported in the Eastern Caribbean and should be included in the list of differential diagnoses for young dogs with gastrointestinal disturbances.

SP-30: SEXUAL DIMORPHISM IN THE EXPRESSION OF ESTROGEN RECEPTOR BETA IN RAT VOCAL FOLD TISSUES

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The sex hormones, especially estrogen, play an integral role in the maintenance of systemic hydration. This study's purpose was to explore the inherent differences between male and female rats in estrogen receptor beta expression throughout the vocal fold and related structures within the larynx. This was accomplished by obtaining coronal sections of laryngeal tissues of ten female and six male rats, followed by immunohistochemistry staining for estrogen receptor beta throughout the sections. These areas were analyzed using digital slide analysis software to determine the percentage of positive staining for each identified area of interest. The areas chosen included true vocal fold epithelium, true vocal fold lamina propria, true vocal fold muscle, subepiglottic and submucosal glands within a single section. The results are pending statistical analysis however the raw data indicates that male rats have a lower level of expression of estrogen receptor beta in the true vocal fold epithelium based on the percentage of positive staining compared to females. There was no large variation between the percentage of strong positive staining in the lamina propria between males and females however females had more total positive staining. Within the true vocal fold muscle males had a higher percentage of strong positive staining. Within the subepiglottic and submucosal glands there was no large difference seen in the positive staining. These results indicate that there may be a difference in the level of expression of estrogen receptor beta throughout the vocal fold which may impact the response these structures have to dehydration challenges.

SP-31: UNUSUAL DISSEMINATED MUCORMYCOSIS IN FERRET

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A 1-year-old spayed female Mustelidae Ferret presented to the Cornell College of Veterinary Medicine Teaching Hospital with a history of anorexia, lethargy and hyperthermia. Abdominal ultrasound revealed two distinct masses occupying the caudal abdomen. Cytology of the abdominal fluid revealed large numbers of hyphae with non-

parallel walls, rare branching, and few septae. Two weeks later, the patient returned to the clinic with hind limb paresis. The patient died and was submitted to necropsy. Necropsy revealed severe, multifocal to coalescing, chronic pyogranulomatous peritonitis, severe diffuse splenomegaly, and moderate subacute hydronephrosis of the left kidney. Histologically, the abdominal masses were composed of extensive areas of necrosis interspersed with large numbers of pauciseptate fungal hyphae with irregular random branching and tortuous walls. Additionally, intralesional mucormycete hyphae were noted in the liver, gallbladder, urinary bladder, lung, small intestine, and left kidney. Changes in the spleen were consistent with atypical myeloid hyperplasia and focal area of acute infarction. Panfungal PCR and sequencing identified *Lichtheimia corymbifera* as the cause. This agent is commonly found in soil or decaying grass. It has been reported to cause secondary otitis externa in ferrets but has not been described as the causative agent of systemic disease in ferrets.

SP-32: MACROPHAGES AND PROSTATE CANCER RACIAL DISPARITIES

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Prostate cancer (PCa) is the most commonly diagnosed cancer in American men. African American (AA) men have 76% higher incidence and 2.3 times higher mortality rates than European American (EA) men. Genetic alterations, African ancestry, increased inflammation-related gene expression, and increased innate immune cells in the tumor microenvironment are proposed contributors to PCa aggressiveness in AA men. Studies suggest that cancer cells secrete factors that change innate immune macrophages from a tumor-inhibiting (M1) to a tumor-promoting (M2) phenotype. In the current study, we evaluated CD163 (M2 marker), CD80 (M1 marker) and CD68 expression in whole tissues sections from radical prostatectomy PCa specimens (n= 19 AA; n=19 EA) and organ donor normal prostates (n=3 AA; n=6 EA) using RNA *in situ* Hybridization (RISH). The HALO® image analysis platform was used to estimate the area of positive signal per unit tissue area. Results indicate a significant increase in $CD163^+$ and $CD68^+$ macrophages in normal prostate tissues compared to PCa tissues ($p<0.05$), particularly in $CD163^+$ macrophages among AA men ($p<0.04$). We found a higher M1/M2 macrophage ratio in PCa compared to donor tissue ($p<0.001$) corresponding to CD163 expression. Interestingly, the mean CD163 expression of normal prostates trended higher in AA compared to EA men, while $CD163^+$ macrophages trended lower in PCa tissues from AA men compared to EA men. Our findings suggest different macrophage patterns in the prostate microenvironment of both normal and PCa tissues from AA versus EA men. Future studies will investigate whether these differences contribute to disparities in PCa outcomes.

SP-33: EQUINE LIMBUS SQUAMOUS CELL CARCINOMA: CASE REPORT

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A 13-year-old, quarter/paint, recreational riding mare from Northern California presented with a very small nodule on the left eye. The mass was found bulging from the cornea and locally effacing the medial limbus. The owner reported progressive growth. A biopsy was taken. A diagnosis of limbal squamous cell carcinoma (SCC) was made. Six months later, the mass had increased in size and began to protrude, covering the cornea and impeding eyesight. The owner elected for enucleation. A second set of biopsies from the enucleated eye were submitted for histopathology. In equine, SCC is one of the most common neoplastic ocular tumors with the cornea being a frequent location. Surgical excision of the ocular SCC is successful if combined with other treatments such as local chemotherapy, cryotherapy, or hyperthermia therapy. Enucleation in this mare was successful with no recurrence of SCC. The development of SCC is suspected to be attributed to exposure to UV radiation. In addition, a genetic risk factor for SCC has been proposed. In this case, the mare had a blaze marking on her face. This hair pattern is noticeably wider towards the left side of the face, encircling the affected eye. It is possible that the lighter pigmentation in this area of the mare's skin could have predisposed the mare for the development of this neoplastic lesion.

SP-34: SPONTANEOUS NEOPLASMS IN RED-BELLIED LEMURS (Eulemur rubriventer)

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Background: Red-bellied lemurs (genus *Eulemur rubriventer*, family Lemuridae, Suborder Strepsirrhini) are non-human primates endemic to the forests of Madagascar and are listed as “vulnerable” on the IUCN Red List of Threatened Species. Currently, reports and characterization of the neoplastic process in this species are extremely scarce, with solely one reported case of hepatocellular carcinoma in the literature to date. **Objective:** Determine the incidence and classify the spontaneous neoplasms in captive red-bellied lemurs. **Methods:** Prosimian submissions received by the Anatomic Pathology Service at the North Carolina State University from January 2010 to January 2021 were retrieved. **Results:** A total of 200 cases of Strepsirrhini prosimians were identified, representing 57 (28.5%) individuals from the *Eulemur* spp. A total of 7 out of 57 (12.3%) cases were red-bellied lemurs. Neoplastic proliferation was identified in 2 out of 7 (28.57%) cases. The first case was an adult female red-bellied lemur of an unknown age with hepatocellular carcinoma. The second case was a 33-year-old, female red-bellied lemur with cervical skeletal osteosarcoma. Hepatocellular neoplasms are commonly reported in prosimians, with carcinomas more frequent than adenomas. In contrast, osteosarcoma is rarely described in lemuriformes, with only two cases reported in the literature: one sacrum osteosarcoma in a Coquerel’s giant mouse lemur *(Mirza coquereli);* and one arising from the carpus and radius of a ring-tailed lemur

(*Lemur catta)*. **Conclusion:** To our knowledge, this is the first report of osteosarcoma and the second description of hepatocellular carcinoma in *E. rubriventer*.

SP-35: ADRENOCORTICAL CARCINOMA WITH PULMONARY MESTASTASES AND PNEUMOTHORAX IN A HYPERTHYROID CAT

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A 15-year-old, female, neutered, domestic short-haired cat with a one-year history of hyperthyroidism was presented for lethargy, tachypnea, and open-mouth breathing. Imaging revealed pneumothorax, several lung nodules and a mass-like lesion enveloping the right kidney. On CT, the right kidney was replaced by an amorphous, non-contrast enhancing mass surrounded by a rim-enhancing, fluid filled sac. Cytology of the perirenal mass revealed an epithelial population compatible with an adrenal carcinoma. The cat deteriorated and was euthanized. Post-mortem examination revealed enlarged thyroid glands, and a soft tissue mass effacing, and likely arising from the right adrenal gland, and surrounding the kidney. Metastases were present in the lungs. Histologically, the neoplasia was composed of pleomorphic polygonal cells forming irregular clusters, cords and weak tubular structures. Cells were immunopositive for cytokeratin, and negative to S100, synaptophysin, and chromogranin A, suggestive of an adrenocortical carcinoma. Primary adrenocortical carcinomas are rare in cats, with the majority being functional tumors. This case report illustrates the unusual manifestation of an adrenocortical carcinoma in a cat with concurrent thyroid adenoma which was presented for metastases-related spontaneous pneumothorax. The imaging and gross presentation of this adrenal tumor was unusual, in addition the cat was not manifesting clinical signs associated with adrenal hormonal secretion. Secondary spontaneous pneumothorax (SSP) has been recorded in cats with pulmonary metastatic neoplasia, which was considered likely a sequela in this case.

SP-36: BICUSPID AORTIC VALVE WITH AORTIC STENOSIS IN AN ADULT REINDEER (RANGIFER TARANDUS)

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A 6-year-old male reindeer (Rangifer tarandus) presented to Cornell University Hospital for Animals with a 2-month history of lethargy, weight loss, and anemia. Babesiosis had been previously diagnosed via blood smear. Physical examination revealed a grade IV/VI systolic heart murmur and subcutaneous edema. Bloodwork was significant for an elevated troponin level (5.88 ug/dL). A tricavitary effusion was diagnosed based on ultrasound. The animal was euthanized and submitted for necropsy. The gross

examination revealed submandibular edema and confirmed tricavitary effusion with fibrin covering the surface of the heart and lungs. The lungs were edematous. The aortic valve was composed of 2 leaflets and the associated chordae tendineae were thickened. Histologic examination confirmed pulmonary edema and revealed hemosiderosis of the lungs, kidneys, and liver . There findings were interpreted as left and right sided heart failure, secondary to a bicuspid aortic valve, exacerbated by babesia-associated anemia. There are limited reports of bicuspid aortic valves in veterinary medicine. Based on the characterization used in humans, this reindeer appears to have an antero-posterior bicuspid aortic valve with a raphe. In humans, clinical disease rarely presents before adulthood, similar to the late onset in this case.

SP-37: CASE REPORT: PRIMARY NEUROLYMPHOMATOSIS IN A DOG

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Background: Neurolymphomatosis is a rare condition characterized by invasion of malignant lymphocytes into peripheral nerves. It is suspected to be a manifestation of lymphosarcoma and can involve either B or T lymphocytes. Objective: To describe the findings of an adult mix-breed canine diagnosed post-mortem with a neurotropic lymphosarcoma. Methods: An 11-year-old, male castrated, mixed breed dog was presented for difficult and painful walking. A full neurologic evaluation was performed. Magnetic resonance imaging (MRI) of the thoracolumbar spine was performed. Cerebrospinal fluid was submitted for cytology. Subsequently, a gross necropsy examination was performed, and tissues were prepared for microscopic evaluation. Immunohistochemical stains CD3 and CD79 were used to further characterize the lesion. Results: A painful and progressive peripheral polyneuropathy was identified on physical examination. Cytology of the cerebrospinal fluid revealed a lymphocytic pleocytosis. MRI showed thickening and hyperintensity of the lumbar nerve roots and sciatic nerves. Relevant gross findings include accumulation of opaque white fluid in the meninges and a soft tan splenic mass. Microscopic findings revealed small lymphocyte infiltrates into spinal and sciatic nerves, along the lumbar dura, and in the meninges. Neoplastic small lymphocytes were confined to neural tissues. Degenerative changes in the sciatic and lumbar spinal nerves were severe. Lymphocytes were diffusely positive for CD3 and negative CD79a. Additionally, a splenic histiocytic sarcoma was identified. Conclusion: This case report explores a unique manifestation of lymphosarcoma with likely primary peripheral nerve involvement. Neurolymphomatosis is rarely described in veterinary species; thus, it is helpful to further characterize the condition.

SP-38: EXPRESSION OF CXCL10, INTERFERON-GAMMA, AND MXA IN CANINE EXFOLIATIVE CUTANEOUS LUPUS ERYTHEMATOSUS

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Canine cutaneous lupus erythematosus (CLE) is an autoimmune skin inflammatory disease characterized by a dense, lymphocytic interface dermatitis and periadnexal lymphocytic infiltration. Within the umbrella of CLE, there are divisions of variants including acute, subacute, and chronic [exfoliative cutaneous lupus erythematosus (ECLE)] forms. Type I interferons (IFN) enhance the cytotoxic ability of circulating lymphocytes and, therefore, induce the production of proinflammatory chemokines and proteins including C-X-C Motif Chemokine Ligand (CxCL) 10, for lymphocyte recruitment, interferon-gamma (IFN-g), and antiviral-Myxovirus protein A (MxA), a marker specific for type I IFN activation. Currently, little is known about the pathophysiological mechanisms of the inflammatory response in canine CLE skin lesions; therefore, the role of the interferon pathway in canine CLE is being investigated using clinically healthy and diseased tissue samples to compare chemokine and interferon protein upregulation via immunohistochemistry. Five clinically healthy thoracic skin samples and eight samples diagnosed with ECLE were evaluated for IFN-g, CxCL10 and MxA expression via immunohistochemistry. Epidermis and follicles of healthy thoracic skin had absent to mild immunopositivity for all three antigens. In canine ECLE, 7/8 samples had mild to severe epidermal and follicular staining for all three antigens. Immunopositivity in the dermal inflammation varied from absent to moderate for CxCL10, absent to severe for IFN-g and moderate to severe for MxA. Overall, epidermal, follicular, and dermal expression of MxA was most severe. Findings support a role of Type I IFN activation in the development of ECLE.

SP-39: VERTICAL TRANSMISSION OF AQUATIC BIRD BORNAVIRUS-1 (ABBV-1) IN PEKIN DUCKS

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Background: Aquatic bird bornavirus-1 (ABBV-1) is highly prevalent in wild waterfowl in North America, causing persistent infection of the nervous system. The main route of transmission is unknown and there is no conclusive evidence for vertical transmission. **Objective:** Investigate vertical transmission of ABBV-1 in Pekin ducks. **Methods:** Day-old Peking ducks were inoculated intracranially with ABBV-1 (IC, n=14) or with carrier alone (control, n=14). Ducks developed and laid eggs until 20 weeks post infection (wpi), when they were euthanized and necropsied. Eggs were collected daily from each group and incubated for 18-21 days. Total RNA was extracted from tissues of adult ducks and from the brain and/or visceral organs of each embryo. Vertical transmission was evaluated by comparing the infectious status of the embryos with that of the adult ducks, assessed by RT-qPCR. **Results:** In the IC group at 20 wpi, 14/14 brains, 9/10 ovaries, and 3/4 testes from the adult ducks were positive for ABBV-1; controls were negative. Microscopically, gonads collected were mature with no lesions. From the control and IC groups, 38 and 27 fertile eggs were collected with peaks of 6 and 4 eggs/day, respectively. In the IC group, there were 7 consecutive days with at least 2 eggs/day. All embryos were negative for ABBV-1. **Conclusions:** All adults were persistently infected with high frequency of gonadal infection. Laying patterns indicate that some eggs were derived from females with infected ovaries. Although egg

parentage was not tracked, these results suggest that vertical transmission of ABBV-1 is highly unlikely.

SP-40: PRESUMED SOLITARY OCULAR LYMPHOSARCOMA (PSOL) IN A 9-MONTH-OLD CAT.

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Presumed solitary ocular lymphosarcoma (PSOL) has been defined as an absence of systemic disease but confirmed presence of ocular lymphosarcoma, either of the conjunctiva or intraocular tissues. PSOL is generally considered rare in cats, dogs, and humans, but should remain a differential for patients with uveitis or conjunctivitis, as reports of frequency of PSOL in the literature are conflicting and these seem to be commonly associated clinical signs. The veterinary-adapted WHO classification scheme further subdivides lymphosarcoma (LSA) based on location into stages I - V. Conjunctival LSA is considered stage I, as it is a part of the mucosa-associated lymphoid tissue, while intraocular manifestations of LSA are often classed as stage V disease, as the eye is typically considered devoid of lymphatic vessels and not a primary lymphoid organ. Recent studies have produced evidence supporting the existence of lymphatic channels in the normal human, murine, ovine, and canine anterior uveal tissues. This has implications for the understanding of intraocular tumorigenesis, tumor biology, and tumor lymphangiogenesis, and it may suggest the need to revisit current classification strategies for ocular LSA. In the current case report, we describe findings from a 9-month-old, female spayed, domestic shorthair cat presenting with a rare T-cell-rich, large B-cell PSOL. Histopathology shows evidence of vessels in the anterior uveal tract that are consistent with lymphatics. This may suggest that intraocular forms of PSOL instead represent a manifestation of stage I LSA, which may carry a better long-term prognosis. Additional investigation into feline ocular lymphatics is warranted.

SP-41: OSTEOSARCOMA EXOSOMES SELECTIVELY HOME TO THE LUNG AND ELICIT PRO-TUMORIGENIC CHANGES IN RESIDENT LUNG CELL POPULATIONS

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Osteosarcoma (OS), the most common primary malignant tumor of bone, often progresses to a highly fatal metastatic disease with limited treatment options. Following resection of the primary tumor, one-third of OS patients relapse with metastases, almost exclusively in the lung. Metastasis is preceded by the formation of a pre-metastatic niche, a process by which distant sites in the body are “primed” for tumor cell seeding by factors secreted by the primary tumor. Of these secreted factors, nano-sized extracellular vesicles, also known as exosomes, have been shown to mediate pre-metastatic changes in resident cells, and display highly specific organotropism in certain metastatic cancers. Little is known about the role of OS exosomes in modulating the

pulmonary microenvironment during OS metastasis. We hypothesize that OS exosomes selectively home to the lung and instruct resident cells to create a pro-metastatic microenvironment characterized by inflammatory and structural changes. To investigate this, we evaluated human OS exosome biodistribution and cellular uptake in mice using intravital imaging, flow cytometry, and immunofluorescence. We also investigated the immunological effects of OS exosomes in vivo in mice and in primary human donor-derived lung fibroblasts and alveolar macrophages. We show that OS exosomes selectively track to the lung and elicit distinct changes in tumor-promoting cytokines in vivo and in vitro. Our findings demonstrate that OS exosomes can alter the lung microenvironment prior to circulating tumor cell arrival. These pro-tumorigenic changes may promote metastasis during OS and could serve as early indicators and potential therapeutic targets for patients with metastatic disease.

SP-42: NOVEL MUTATION ASSOCIATED WITH GLOBOID CELL LEUKODYSTROPHY IN A FAMILY OF BORDER COLLIE MIX DOGS

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Krabbe's Disease, also called Globoid Cell Leukodystrophy (GCL), is a fatal autosomal recessive disease caused by mutations in the hydrolaseβ-galactocerebrosidase (GALC) gene that results in death within the first two to five years of life of humans, dogs, and many other species. Clinical signs include progressive ataxia, head tremors, and incontinence. Histologically, the characteristic globoid cell, a plump, often multinucleated macrophage containing positive PAS-staining cytoplasmic contents, can be found along white matter tracts, along the gray/white matter junction in the brain, and in perivascular spaces in the brain and spinal cord. There are many reported causative mutations for GCL in humans, but only a few breed-specific mutations have been characterized in canines. Several cases of GCL were diagnosed in a family of Border Collie mixes via the presence of globoid cells on histopathology. Sequencing of GALC in the affected dogs revealed that the previously reported canine mutations were not present. Instead, a novel missense mutation was identified via Sanger sequencing that was homozygous in all affected animals. The GALC gene was characterized in thirty-seven dogs from the family of the affected animals as well as five unrelated dogs. Seven dogs were homozygous for the mutation, including those confirmed to have GCL on necropsy. Seven dogs were heterozygous carriers for the mutation. The mutated allele was absent in the remaining twenty-eight dogs tested, including the unrelated dogs. Determination of allele frequency in a wider population of Border Collies and related breeds is indicated.

SP-43: MUCOPOLYSACCHARIDOSIS I IN A MIXED BREED PUPPY

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A 7-month old, intact male, Chihuahua/Yorkie mix was presented to the University of Missouri for respiratory distress and hindlimb paraparesis. On examination, the patient had dull mentation alongside tetraparesis and generalized proprioceptive ataxia, especially in the hindlimbs. Musculoskeletal abnormalities included a disproportionately large head, abnormal ribs and spine, and dropped carpi and tarsi. Stridor and bilateral corneal opacities were also observed. Radiographs revealed diffuse osteopenia, kyphosis, misshapen vertebrae, hypoplastic trachea, synostosis of the 5th-6th ribs and T5-T6, intracapsular swelling and valgus curvature of the stifle, misshapen tarsal bones, and abnormal epiphyses of the proximal tibia and distal femur. CBC results included mild mature neutrophilia, lymphocytosis, and monocytosis. Peripheral leukocytes contained moderate to abundant amounts of fine, round to ovoid, magenta cytoplasmic granules, and occasionally clear vacuoles. Findings were consistent with lysosomal storage disease, presumptively a mucopolysaccharidosis. The patient was euthanized and a necropsy performed, revealing kyphosis of the thoracic spine and axonal degeneration within the spinal cord. Additionally, small unstained vacuoles were present within the cytoplasm of pyramidal cells and spinal motor neurons. Samples were submitted to PennGen for enzymatic activity quantification, which revealed 0% activity in α-L-iduronidase compared to the control, confirming a diagnosis of mucopolysaccharidosis (MPS) I. Mucopolysaccharidoses are inherited metabolic disorders in which one or multiple lysosomal enzymes are deficient, resulting in accumulation of glycosaminoglycans and multi-systemic disease. These conditions are rare and the prognosis grave. Diagnosis is aided by observation of characteristic abnormalities within leukocytes and confirmatory genetic testing.

SP-44: PATHOGENIC BACTERIAL SPECIES IN PORCINE HEARTS: IDENTIFYING SOURCES OF ENVIRONMENTAL CONTAMINATION FOR THE DEVELOPMENT OF SUPERIOR TISSUE COLLECTION PROTOCOLS

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Background: Microbial contamination is a barrier for medical professionals seeking animal tissue from reputable sources. A local tissue sourcing business provides swine hearts to a research laboratory. Swine are raised on a specific farm, under standards deemed acceptable for research purposes. Recent heart samples collected at a local slaughterhouse from these animals revealed higher-than-average numbers of colony forming units. These large “bioburden counts” on the hearts make them unsuitable for research. Identifying the source of this contamination is an important step towards being able to provide safe samples for research. **Objective:** The study goal is to identify sources of contamination on biomedical porcine heart samples within an Indiana slaughter facility so that microbial contaminants are minimized. **Methods:** Sterile swabs of knives, gloves, collection pans, and the nasal and oral cavities of swine are taken before, during, and after slaughter procedures. The swabs are then labelled appropriately and transported to Purdue University for further processing. Within the lab, cotton tipped applicators are streaked onto TSA agar plates and placed in the incubators. The plates are incubated for 14 days total: 10 days in the incubator and 4 days in room temperature. Culturing methods are adapted from specific protocols used

by the clientele of the tissue sourcing company. **Results and Conclusion:** The findings of this study allow the specific slaughterhouse to implement better practices to decrease contamination levels of their product. The researchers receiving the tissues are able to conduct their work with a high level of confidence in their results.

SP-45: OSTEOCHONDROMATOSIS CAUSING PROGRESSIVE PARAPARESIS IN A PEDIATRIC GERMAN SHEPHERD DOG

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A 4-month-old German Shepard dog presented for non-ambulatory paraparesis progressing to left hind limb monoplegia. Magnetic resonance imaging revealed the presence of three bony proliferations extending into the vertebral canal at the level of C5, L3, and L5. Osteochondromatosis or multiple cartilaginous exostoses was suspected, and the dog was euthanized due to poor surgical options and grave prognosis. At autopsy, smooth hard masses arising from the vertebra were identified which correlated perfectly with antemortem imaging. Masses were characterized by a smooth cartilage cap which, on cross section, exhibited complete and orderly transition to woven bone. Similar bony masses were identified on several ribs, long bones, left scapula and multiple digits. Histologically, the masses were sessile proliferations of hyaline cartilage which transitioned to woven bone through complete and orderly endochondral ossification. In areas of spinal cord compression, approximately 70% of the white matter exhibited segmental Wallerian degeneration with spheroids, ellipsoids, and digestion chambers. Incidentally, the dog was unilateral cryptorchid. Osteochondromatosis is a rare, endochondral proliferative disorder which typically presents during skeletal development in growing people, horses, and dogs. However, in cats, osteochondromatosis occurs after skeletal maturity. These bony outgrowths (exostoses) are generally benign and stop growing with skeletal maturity; however, malignant transformation into osteosarcoma or chondrosarcoma can occur. In dogs, the polyostotic form is an inherited autosomal dominant genetic disorder and is likely caused by a loss of function mutation in the exostosin 2 (EXT2) gene. Loss of function in EXT1 or EXT2 is reported in >90% of human cases.

SP-46: Characterization of CD3+/CD20+ Large Cell Canine Lymphoma

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Introduction: Immunophenotyping of canine non-Hodgkin's lymphoma for B-cell and T-cell surface antigens is commonly performed to better elucidate the clinical outcome or potential treatment options. Expression of CD3 is associated with T-cell malignancies while CD20 is expressed in B-cells. However, a small subset of canine non-Hodgkin lymphomas express both CD3 and CD20 ($CD3^+/CD20^+$). Currently, this form of non-Hodgkin lymphoma remains poorly defined at the molecular level. **Objective**: In this retrospective study, we aimed to better characterize the histogenesis of $CD3^+/CD20^+$ lymphoma. Formalin fixed paraffin embedded tissues from ten cases of $CD3^+/CD20^+$

large cell lymphoma and breed-matched controls of peripheral large T-cell lymphoma and diffuse large B-cell lymphoma were selected from the Michigan State University Veterinary Diagnostic Lab. **Results**: Using PARR, we identified monoclonal T-cell receptor (TCR) rearrangements in all $CD3^+/CD20^+$ cases. In addition, three out of ten cases also exhibited monoclonal rearrangements in the immunoglobulin heavy chain (IgH), supportive of dual lineage rearrangement. There was no significant difference in frequency of antigen receptor rearrangement between $CD3^+/CD20^+$ and $CD3^+$ cases. In comparison with $CD20^+$ lymphomas, $CD3^+/CD20^+$ lymphoma exhibited TCR rearrangement more frequently and IgH rearrangement less frequently ($p=0.0007$ and 0.003, respectively). Immunohistochemical staining of the B-cell marker PAX5 was negative in all $CD3^+/CD20^+$ cases. **Conclusions**: Cases of canine $CD3^+/CD20^+$ demonstrate similar antigen receptor rearrangements and PAX5 staining properties when compared with CD3+ lymphomas, suggesting a similar histogenesis of these two subsets of lymphoma.

SP-47: AVIAN POLYOMAVIRUS WITH SEVERE MACRORHABDUS SPP. INFECTION IN TWO ADULT BUDGERIGARS (MELOPSITTACUS UNDULATUS)

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Formalin fixed tissues from two adult parakeets were submitted for histopathology. The liver and kidneys of both birds had multiple karyomegalic cells with ovoid, finely granulated, basophilic to amphophilic glassy intranuclear inclusion bodies that peripheralized the chromatin. Additionally, the lumen and mucosa of the proventriculus, isthmus, and proximal ventriculus contained numerous basophilic rod-shaped yeast organisms consistent with *Macrorhabdus ornithogaster*. The large intranuclear inclusion bodies seen within the kidney and liver are most consistent with avian polyomavirus also known as Budgerigar Fledgling Disease. Avian polyomavirus belongs to the Papovavirus family of nonenveloped icosahedral viruses that contain circular double stranded DNA. This virus is a highly contagious and infectious disease that can be transmitted horizontally through feather dust, regurgitated food, respiratory and urinary excretions, and reports within budgerigars document vertical transmission. This disease primarily affects psittacines with budgerigars of approximately 1-3 weeks of age having the highest mortality rate. Fledglings develop acute clinical signs such as lethargy, crop stasis, and death within 24-48 hours. Adults are fairly resistant to disease but can seroconvert allowing for the virus to be shed for up to 90 days prior to clearing the infection. Macrorhabdus ornithogaster is an intestinal yeast organism commonly seen in budgerigars. It is a highly contagious disease though not all birds will be clinical for the disease; however, commonly reported clinical signs include weight loss, diarrhea, regurgitation.

SP-48: EPIDURAL MYELOLIPOMA IN A SILKEN WINDHOUND

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A twelve-year old spayed Silken Windhound presented to the University of Missouri Veterinary Health Center for generalized lethargy, muscle atrophy and lumbosacral pain. Physical exam was unremarkable, CBC and chemistry findings were within reference intervals and neurologic exam localized pain to both lumbosacral and cervical regions of the spine. MRI showed an extradural mass of hypointensity over the body of L7. Client elected for a L7-S1 dorsal laminectomy where a pale brown mass integrated into the epidural fat was excised. Impression smears were submitted for cytology and the mass for histopathology. Cytology was highly cellular and consisted of a mixed population of hematopoietic cells on a bloody and lipid-laden background. Histopathology described approximately 60% hematopoietic cells and 40% adipose tissue; a spinal myelolipoma was diagnosed. The patient made a full recovery, and, at the time this report is written, the dog's neurologic signs are resolved. Myelolipomas are benign tumors consisting of hematopoietic precursors interspersed in fat. These tumors are rarely found in domestic animals and are most commonly diagnosed in the liver, spleen, and adrenal glands. There have been three case reports in the veterinary literature of epidural myelolipomas, and all three reports were from senior male sled dogs. Only one of these patients underwent MRI and treatment. This case represents the first report of an epidural myelolipoma in a sighthound breed. Additionally, it provides multiple magnetic resonance sequences earlier in disease progression than previously shown with corresponding cytological and histopathological findings, and short-term outcome post-surgical excision.

SP-49: BOVINE TUBERCULOSIS IN AFRICAN BUFFALO (SYNCERUS CAFFER): PROGRESSION OF PATHOLOGY DURING INFECTION AND CORRELATION TO GENETIC MARKERS

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Background: Bovine tuberculosis (BTB) is a zoonotic disease of global importance that is endemic in sub-Saharan Africa, including Kruger National Park where the study took place. **Objective:** We first sought to describe the pathologic progression of bovine tuberculosis over time in infected African buffalo. We then asked if progression correlates to genotypic markers. **Methods:** African buffalo were captured, fitted with a tracking collar and tested for BTB during a 4-year-cohort study. This allowed for serial captures and BTB testing for the buffalo in the study, creating a database with known sero-conversion dates for those not infected with BTB at initial capture. At the end of the project, BTB positive buffalo were culled, and necropsies completed. **Results and Conclusions:** We found that as the infection progressed, more lung lobes were

affected with bigger lesions, as opposed to a single lobe with bigger lesions. There is a weak positive correlation between disease progression with length of infection, reinforcing the difficulty in developing a clear timeline for bovine tuberculosis. The probabilities of retropharyngeal and tracheobronchial lymph nodes infection mirror each other, with high likelihood of pathology early in the disease process. Lastly, the previously identified BTB risk allele, SNP 3195, is of particular interest in terms of progression of lung necrosis. Lung lesions from buffalo with the homozygote genotype are less necrotic when controlled for length of infection, compared to wildtype and heterozygote genotypes. This could suggest the homozygote genotype serves a protective function within this herd.

SP-50: Investigating diagnostic utility of agarose cell blocks prepared from hepatic and splenic aspirates

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Cytologic evaluation of fine needle aspirates is an inexpensive and minimally invasive diagnostic technique, but advanced testing (e.g immunocytochemistry) on these samples is limited by small and unevenly distributed samples and lack of control materials. Cell blocks are prepared by embedding aspirates into a solid matrix, which can then be routinely fixed and processed in the histopathology lab. The aims of this study were to determine whether 1) cell blocks of adequate cellularity can be prepared from hepatic and splenic aspirates, and 2) samples can be stored for 24 or 72 hours prior to cell block preparation. Aspirates from canine cadavers were either expelled directly into warmed liquid agarose, or into one of five storage media: Hanks buffered saline solution (HBSS), HBSS + 5% albumin, 1 HBSS + 10% albumin, fetal bovine serum, or canine serum. Samples were refrigerated for 24 and 72 hrs, and then embedded into agarose, formalin-fixed, paraffin-embedded, and sectioned for H&E and immunohistochemistry (IHC). Cell blocks prepared from fresh hepatic and splenic aspirates yielded high cellularity specimens with excellent cellular morphology. IHC for cytokeratin, vimentin, CD3, Pax5, and Iba-1 yielded expected results with minimal background. In stored samples, protein in the media improved cellular preservation compared to storage in HBSS alone. We conclude that agarose cell blocks can be prepared from aspirates of solid tissues, and that aspirates can be stored for up to 72 hours prior to preparation. These findings expand the potential of utility of cell blocks beyond academic veterinary institutions.

SP-51: ASSESSING HISTOPATHOLOGY TECHNIQUES FOR SOFTENING CLAW SPECIMENS

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Background: Claws are particularly difficult to process for histopathological analysis. Literature on the processing of claws is sparse, though some labs use 15% formic acid solution. However, this technique has potential to denature nucleic acids and proteins, which may impact ancillary studies. The study objective was to examine alternative claw

softening techniques. **Methods**: Five solutions were tested; 17% potassium hydroxide solution, 10% potassium thioglycolate, fabric conditioner, a formic acid and hydrochloric acid-based decalcification solution, and 15% formic acid solution. Healthy claws from dogs were fixed in 10% formalin before soaking in softening solutions. Paramedian longitudinal cuts were made into the claw and sections were routinely stained with hematoxylin and eosin. **Results**: Claws were soaked for 4 hours to 27 days until soft enough to slice with a trimming blade. All claws were paraffin embedded and cut with a steel microtome except for the specimen soaked in fabric conditioner, which remained too hard to process. The claw soaked in 17% potassium hydroxide softened most rapidly (4-5 hours) maintaining histologic integrity, which was lost at 7 days. 10% potassium thioglycolate required 7 days for softening but lacked histologic structural integrity. The formic acid and hydrochloric acid-based, and 15% formic acid-based decalcification solutions required 7 and 27 days, respectively, and maintained histologic integrity. **Conclusion**: Formic acid-based solutions are appropriate for routine histologic processing of claws. 17% potassium hydroxide solution can be utilized as an alternative solution to allow for ancillary tests but must be used judiciously due to the speed of nail softening.

SP-52: NOVEL DIAGNOSTICS FOR SEPTIC EFFUSIONS: OPTIMIZING FOUNTAIN FLOW CYTOMETRY AND TESTING THE RAPIDBAC VET IMMUNOASSAY

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Bacterial sepsis is a life-threatening condition in which rapid diagnosis is critical for survival. The current primary diagnostic tools for identifying septic cavity effusions are bacterial culture (slow turnaround time) and fluid cytology (suffers from low sensitivity). Fountain Flow™ Cytometry (FFC) is a tool designed to enumerate microbes at low concentrations in municipal and commercial fluids. A preliminary FFC diagnostic trial conducted by our laboratory on cavity fluids from 60 septic mammalian patients demonstrated a sensitivity and specificity of 60% and 94.4%, respectively. The RapidBac™ Vet (RBV) test is an immunoassay designed for point-of-care detection of bacteria in small animal urine. Both FFC and the RBV test are inexpensive and quick to perform. The aim of this study was to both optimize the use of FFC and to assess the capabilities of the RBV test, for detection of septic effusions. Sterile cavity effusions from mammalian patients were spiked with known quantities of bacteria and then analyzed by FFC. Significant losses of bacteria were observed (32.4%-97.7%) and determined to be due to sample preparation methods, also potentially accounting for the previously observed low sensitivity. A case control study (n=16) of the RBV test using 1-2 day-old, refrigerated mammalian cavity effusions yielded a sensitivity and specificity of 100% and 44.4%, respectively, indicating false positives may be problematic for RBV use to detect septic effusions, and that a more controlled study involving fresh samples is warranted. FFC offers multiple advantages over currently used methods of septic effusion detection and thus warrants further testing.

SP-53: DUCTAL PLATE MALFORMATION IN A JUVENILE LABRADOR RETRIEVER

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A 2-year-old male neutered Labrador Retriever had surgical repair of a peritoneal-pericardial diaphragmatic hernia at Mississippi State University College of Veterinary Medicine. Prior to surgery, the dog had markedly elevated liver enzymes and ascites. During surgery to correct the hernia, multiple acquired portosystemic shunts were noted and liver biopsies were collected. The left medial lobe was markedly small and pale, while the other lobes were slightly firm. Microscopically, the left medial lobe lacked hepatocytes and was composed of bile ducts surrounded by a spindle cell population embedded in a primitive collagenous matrix. Biopsy from a more normal lobe had portal bridging fibrosis with numerous bile duct profiles. Portal tracts had small to absent portal veins with lymphatic dilation. These changes were consistent with hepatic ductal plate malformation (DPM). The dog declined following surgery, and euthanasia was elected due to poor prognosis. At necropsy, the gallbladder and cystic, hepatic, and common bile ducts were moderately dilated. DPMs are developmental anomalies that result from lack of ductal plate remodeling during bile duct morphogenesis. There is a phenotypic spectrum including polycystic liver disease, congenital hepatic fibrosis, and Caroli disease. The histologic changes of congenital hepatic fibrosis are characterized by bridging portal fibrosis with numerous bile ducts, as seen in this case, with the left medial lobe being most severely affected. The dilated cystic, hepatic and common bile ducts may be part of the spectrum of disease. Case reports of congenital hepatic fibrosis in veterinary species are rare, and unreported in Labrador Retrievers.

SP-54: USE OF A DEEP LEARNING ARTIFICIAL INTELLIGENCE MODEL FOR DIFFERENTIATING ALVEOLAR AND BRONCHIOLAR ADENOMAS AND CARCINOMAS IN THE LUNG IN B6C3F1 MICE

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Background: Diagnostic toxicologic pathology is rapidly transitioning to digitalization and automation. The standard is labor-intensive and time-consuming hence, there is a growing need to develop faster automated methods that would reduce costs, improve diagnostic accuracy, consistency, and workflow. Pathologists have begun using new technologies such as artificial intelligence to evaluate toxicologic pathology studies. **Objective**: The objective of this study was to develop and train a computer-assisted artificial intelligence system that could be used to diagnose mouse alveolar/bronchiolar adenoma and carcinoma in the lung. Once trained, the system was tested on a set of known lung tumor diagnoses to assess the accuracy of the artificial intelligence model. **Methods**: Using hand-drawn annotations on the digitized images, a supervised, multi-layered, convoluted neural network algorithm was created and trained to detect and recognize normal and tumor tissue, as well as the histological features or patterns that differentiate adenoma from carcinoma. **Results**: The deep learning algorithm was able to identify and distinguish between background and lung tissue, normal lung from tumor,

and finally adenoma versus carcinoma. **Conclusion**: These results suggest that artificial intelligence could provide a sensitive and objective automated method to screen, detect, and diagnose proliferative lesions in lung sections. However, to increase sensitivity of the algorithm, the size and variability of the training data set and the number of annotations.

SP-55: GENETIC MECHANISMS OF RESISTANCE TO BTK INHIBITION IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is a common, aggressive cancer affecting both humans and dogs, and effective therapies without toxicities are lacking. In a previous study, our laboratory performed a clinical trial to investigate the efficacy of BTK inhibition in canine DLBCL using the BTK inhibitor acalabrutinib. The objective response rate was 25%; thus, most patients demonstrated primary resistance. In the current study, we performed whole exome sequencing of canine lymphoma cells before and after acalabrutinib therapy to discover mutations in major drivers of B-cell cancers that could propagate cell survival and proliferation despite proximal BCR inhibition with acalabrutinib. We identified 242 unique variants affecting 202 different genes, of which 39 were high impact mutations and 203 were moderate impact. On average, there were 21.7 variants (median=21.5 variants). We found that four genes, *CSMD1*, *DDX3X*, *MYC*, and *SETD2*, were more frequently mutated in patients that achieved stable disease, while two genes, *FRAS1* and *ENSCAFG00000030258*, tended to be mutated in patients with a partial response to therapy. Six genes, *MAP3K14*, *CSMD1*, *DDX3X*, *POT1*, *SETD2* and *ENSCAFG00000030258* tended to be mutated in samples of patients that had received chemotherapy prior to initiating acalabrutinib therapy, compared with chemotherapy-naïve dogs. However, due to the small sample size, these findings were not statistically significant, and further investigations must be performed to verify trends. These data will guide future investigations to determine how specific mutations and genes alter molecular pathways driving lymphoma cell survival and drug resistance.

SP-56: DEVELOPMENT OF A DEEP LEARNING METHOD FOR BRONCHOALVEOLAR LAVAGE FLUID (BALF) ANALYSIS

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Bronchoalveolar lavage fluid (BALF) analysis, a commonly performed endpoint in drug discovery and safety assessment studies, provides insight into the pulmonary system, and allows for sampling at multiple timepoints for comprehensive evaluation. However,

this is a time-consuming manual analysis. We hypothesized that a deep learning artificial intelligence (AI) algorithm could be developed to detect, differentiate, and quantify the various cell types (lymphocytes, macrophages, neutrophils, eosinophils) found within BALF with a high level of sensitivity and specificity. Whole slide images (WSI) of prepared BALF samples from control rats and rats administered test compounds were scanned at 40x magnification with an optical zoom of 60x on a Leica AT2 scanner and uploaded to the Deciphex Patholytix Preclinical Study Browser. Individual cellular annotations were performed at 40x magnification for 6 classes defined using the expertise of boarded veterinary clinical pathologists. A convolutional neural network (CNN) model was trained using these annotations to create AI generated masks and perform class-based object counting. Performance was evaluated visually during training and quantified at the pixel level using confusion matrices and F1 scores. Each individual class performed at or above an F1 score of 0.9 on a blinded validation set that was put aside from the training dataset. The model performance was also visually verified by the clinical pathologist for the intended use. The algorithm successfully differentiated between the cell lineages commonly found in BALF and has the potential to provide automated and quantitative decision support for the clinical pathologist.

SP-57: OVARIAN AND UTERINE BACKGROUND AND COMMON HISTOLOGIC LESIONS IN FREE-RANGING XENARTHRANS

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Background: Xenarthra is the superorder of placental mammal's endemic to America, comprising the order Pilosa (anteaters, sloths) and the order Cingulata (armadillos). Comprehensive microscopic analysis of the reproductive tract of Xenarthrans is scarce and, to the best of our knowledge, characterization of the ovarian and uterine background and common lesions in free-ranging Xenarthrans has yet to be described. **Objective:** To describe the histologic ovarian and uterine background lesions in free-ranging Xenarthrans. **Methods:** Three independent Ethical Brazilian committees approved this study. Female reproductive tract was collected during post-mortem examinations from Xenarthrans that died from wildlife-vehicle collisions. **Results:** A total of 8 Xenarthrans belonging to three species were identified: 3 southern anteaters (*Tamandua tetradactyla),* 3 giant anteaters (*Myrmecophaga tridactyla),* and 1 giant armadillo (*Priodontes maximus*). The ovaries were analyzed in 75% of individuals (6/8) with the following findings: follicular cysts 66.7% (4/6) and atretic follicles 33.3% (2/6). Uterine findings (87.5%; 7/8) included mucometra 57.1% (4/7), endometritis 28.6% (2/7), metritis 14.3% (1/7), and endometrial cysts 14.3% (1/7). **Conclusion:** Our research provided the microscopic description of the spontaneous ovarian and uterine background and common lesions in Xenarthrans, which is valuable knowledge for a

better understanding of the female reproductive system and therefore crucial for reproductive management in Xenarthrans in captivity.

SP-58: DISSEMINATED HEMANGIOSARCOMA WITH HEMOABDOMEN IN A HORSE

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Disseminated hemangiosarcoma is poorly described in horses and antemortem diagnosis is challenging due to nonspecific clinical signs. A 22-year-old, Warmblood gelding presented with a one-day history of fever (38.9ºC), colic with impaction on rectal palpation, and marked macrocytic, normochromic regenerative anemia (MCV 55.1, MCHC 38.5, HCT 15%). Abdominal ultrasound showed a 10 cm in diameter, cavitated splenic mass. Given the clinical presentation and poor prognosis, humane euthanasia was elected. Postmortem examination revealed 3 L of frank blood in the abdominal cavity. The spleen had multifocal to coalescing, dark red, semi-firm, raised masses ranging from 0.2 cm diameter to 12 x 8 x 6 cm. Extending from the capsular surface of the left kidney and severely compressing the subjacent renal cortex was a non-encapsulated similar mass associated with subcapsular hematoma (approximately 23 x 7 x 21 cm; 1.1 kg). Similar masses were found in the serosal surface of the small and large intestines, omentum, mesentery, liver, diaphragm, stomach, adrenal glands, kidneys, lymph nodes, peritoneum, extra orbital muscles, epaxial muscle, thoracic pleura, lungs, esophagus, pericardial sac, and all layers of the heart. Microscopically, the masses consisted of non-encapsulated, poorly demarcated, infiltrative, densely cellular neoplasms composed of spindle cells forming irregularly variably sized blood-filled vascular channels, streams, or densely cellular sheets with areas of necrosis, hemorrhage, and organizing thrombi mixed with hemosiderophages and hematoidin. Anisocytosis and anisokaryosis were moderate with 50 mitotic figures in 2.37 mm^2. The gross and microscopic findings were consistent with splenic hemangiosarcoma with disseminated metastases and secondary hemoabdomen.

SP-59: INVESTIGATING THE PREVALENCE OF EQUINE PARVOVIRUS IN ONTARIO HORSES AND ITS ASSOCIATION WITH SERUM MARKERS OF HEPATIC DISEASE

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Equine parvovirus-hepatitis virus (EqPV-H) has been associated with equine serum hepatitis (Theiler's disease), a common cause of acute hepatic failure. The most common source of EqPV-H is administration of equine biological products contaminated with EqPV-H, but non-biological sporadic transmission has been reported. Although most EqPV-H positive horses are asymptomatic or subclinical, some develop clinical signs similar to Theiler's disease, including lethargy, anorexia, icterus, neurological

signs, and/or death. This study investigated the molecular prevalence of EqPV-H and its association with serum markers of liver disease in mares and their foals at a farm in Ontario, Canada as well as the EqPV-H prevalence in horses across Ontario. 11 mares and their foals were sampled twice in 2019, 36 days apart after a mare developed peracute neurological disease associated with Theiler's disease, with 130 additional mares sampled across Ontario. DNA was isolated from serum and tested for EqPV-H using quantitative-PCR. Seroprevalence of EqPV-H DNA on the farm was 77.2%, with 17/22 of horses testing positive at at least one time point, with viral loads ranging from 5.93×10^2 to 6.10×10^5 copies/mL. Serum biochemistry and bile acid panels were performed at the second time point to assess liver disease. Regression analysis between EqPV-H DNA copy number and serum parameters revealed no strong relationship between the presence of parvovirus and indicators of liver disease, with the highest correlation found with GLDH (R^2 of 0.396) and AST (R^2 of 0.123). The role of EqPV-H in Theiler's disease remains uncertain and requires further research.

SP-60: OLFACTORY NEUROBLASTOMA WITH WIDESPREAD METASTASIS

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An 8 year old male castrated Siberian Husky presented to the Internal Medicine Service at the University of Missouri Veterinary Teaching Hospital for chronic nasal discharge, congestion, and periodic epistaxis. The discharge had been partially responsive to prednisone and Convenia therapy. Imaging of the head and thorax via computed tomography (CT) scan revealed a nasal mass effacing the entire left nasal cavity and part of the right nasal cavity. The mass did not extend into the cribriform plate. An abdominal ultrasound demonstrated nodular splenic irregularities and an unidentified nodule adjacent to the spleen. Biopsies were obtained by endoscopy and submitted for histopathology. Aspirates of the liver, spleen, intra-abdominal nodule, and regional lymph nodes were obtained for cytological evaluation. Histopathology revealed a densely cellular infiltrative round cell neoplasm with a high mitotic index. The neoplasm was CD20-, CD3-, Cytokeratin-, Chromogranin A+, NSE+, S100- consistent with a tumor of neuroendocrine origin. The final diagnosis was an olfactory neuroblastoma. Cytological evaluation of the liver, spleen and intra-abdominal mass aspirates revealed low numbers of round cells resembling embryonal tumour cells, consistent with metastasis of the olfactory neuroblastoma. Olfactory neuroblastomas are rare neoplasms that make up about 1% of neoplasias in dogs and arise from the sensory neuroepithelial olfactory mucosa in dogs and cats. The tumour is often difficult to differentiate and requires histopathology for confirmation. There are only a few case reports of metastatic olfactory neuroblastoma in small animals.

SP-61: ECTROMELIA VIRUS C15 PROTEIN FACILITATES PATHOGENESIS IN VIVO BY ANTAGONIZING BOTH THE INNATE AND ADAPTIVE IMMUNE RESPONSES

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Ectromelia virus (ECTV) is amongst the most notorious of laboratory animal pathogens, and its virulence relies upon extensive evasion of the murine immune response. ECTV C15 is an immunomodulatory protein previously identified to antagonize T cell receptor-dependent CD4 T cell responses, but this work identifies a novel second innate immune-inhibitory function of this protein that strongly impacts virulence *in vivo*. By comparing pathogenesis of WT and a C15 deficient ECTV, we found that C15 facilitates viral spread *in vivo* by 3 days post infection, in a manner that depends both on CD8 T cells and NK cells. Further investigation of NK cell function both *ex vivo* and *in vitro* shows that C15 selectively antagonizes degranulation of NK cells but not production of antiviral cytokines. Additionally, imaging of infected tissues illustrates that C15 inhibits the interaction of NK cells with infected cells. This work demonstrates the discovery of a novel second function of the protein, which can selectively antagonize both the innate and adaptive murine immune responses. These results prompt further investigation into the mechanism used by C15 to inhibit these cell types and provides novel evidence that viral targeting of CD8 T cells can impact virulence at very early timepoints.

SP-62: A RETROSPECTIVE STUDY ON THE CAUSES OF MORTALITY OF CHELONIANS IN THE SOUTHEASTERN USA (1976-2018)

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Background: Chelonians are valuable indicators of ecosystem health and have high economic values. However, population declines are ongoing and often are attributed to anthropomorphic and biologic factors. **Objectives:** As published reports on chelonian mortalities are limited, we retrospectively summarized the primary causes of mortality of chelonians submitted to the Southeastern Cooperative Wildlife Disease Study from 1976-2018. We further analyzed the data for demographic, seasonal, geographic, and temporal trends. **Methods:** 221 chelonians (151 aquatic, 70 terrestrial) were included. Diagnoses were achieved through gross (carcasses) and histopathological (carcasses and field-collected tissues) evaluation, with ancillary testing as needed. Causes of mortality were categorized into infectious, non-Infectious, and undetermined and further subcategorized into bacterial, viral, fungal, endoparasitic, trauma, physiologic stress, toxins, and other. **Results:** For aquatic species, mortalities were most often attributed to ulcerative shell disease (37) followed by undetermined (31), infectious (24 bacterial, 12 fungal, 25 endoparasitic, 2 other), and non-infectious (8 trauma, 19 physiologic stress, 3 toxin, 2 other). For terrestrial species, infectious causes of mortality (41 bacterial, 10 viral, 1 fungal) were more common, followed by non-infectious (9 trauma, 3 physiologic stress, 11 other) and undetermined (5). Seasonal trends corresponded to physiologic stress, and bacterial, fungal, and trauma diagnoses. **Conclusion:** Although retrospective studies have inherent sample bias, this study provides a long-term perspective on causes of chelonian mortality in the southeastern USA. Results provide baseline information that can be used for future comparisons and contribute to a better understanding of chelonian conservation and management needs and epidemiologic patterns.

SP-63: PRIMARY FELINE BILIARY CARCINOID MIMICKING AN INTERMEDIATE-CELL LYMPHOMA ON CYTOLOGY

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A 12-year-old, spayed female, domestic short hair cat was presented for evaluation of a one-month history of progressive lethargy and inappetence. On physical exam, the patient was quiet, alert and responsive with marked, generalized icterus. Blood work revealed severe hyperbilirubinemia (22.3 mg/dL; reference interval [RI] 0.10-0.30 mg/dL) with mixed elevated liver enzyme activities, including ALP (292 U/L; RI 10-79), GGT (14 U/L, RI 0-5 U/L), ALT (467 U/L; RI 25-145 U/L), and AST (203 U/L; RI 5-42 U/L). An abdominal ultrasound found a distended gallbladder with a large mass associated with the extrahepatic bile duct. A fine-needle aspirate obtained of the mass revealed variably cohesive clusters of monomorphic mononuclear cells that were intermediate in size with round or oval nuclei and a high nucleus to cytoplasm ratio, suggesting an intermediate-cell lymphoma. Due to poor prognosis and worsening clinical signs, humane euthanasia was elected. On post-mortem examination, the mass was multi-lobular and protruded from the common bile duct. On histopathologic evaluation, the neoplasm was composed of round to columnar cells arranged in packets, consistent with an extrahepatic carcinoid, confirmed by a positive immunohistochemical reaction to Chromogranin A. This rare neoplasm is believed to originate from neuroendocrine cells diffusely scattered throughout the biliary tree. Description of the cytologic appearance of these tumors is scarce in the literature and their variably cohesive and monomorphic appearance, mimicking a lymphoid neoplasm in this case, is of note.

SP-64: MASSIVE BEE ENVENOMATION IN A KANGAROO (MACROPUS SP.)

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Several kangaroos (*Macropus* sp.) presented with a history of being stung by a swarm of honeybees (*Apis mellifera*) in an exhibit, and many died over the 4 days following the event. An adult, female, tan and white kangaroo (*Macropus* sp.) presented to Texas A&M Veterinary Medical Diagnostic Laboratory for necropsy. On necropsy, the animal exhibited marked facial swelling, especially over the right mandible. Other findings included icterus, multifocal to coalescing red to dark red areas in the lungs, and a diffusely yellow liver with an accentuated reticular pattern. The main histologic findings consisted of multifocal necrosis in the myocardium, acute tubular necrosis in the kidney with intratubular pigment (hemoglobin) and protein, and multifocal hepatocellular necrosis. Additionally, all three organs had evidence of hemorrhage and edema. Though the histopathologic findings are not specific, they support a diagnosis of fatal massive bee envenomation. Death was likely due to multiorgan dysfunction syndrome, which resulted from a combination of the toxicity of the venom, intravascular hemolysis due to bee hemolytic factors, systemic microangiopathy, and severe hypotension due to

anaphylactic shock. To the best of our knowledge, this represents the first description of fatal bee envenomation in macropodids. The diagnosis of massive bee envenomation is one of exclusion and should stem from a history of suspected or confirmed exposure to numerous bees combined with the onset of appropriate clinical signs and compatible pathologic findings.

SP-65: PUTATIVE PERILLA FRUTESCENS (L.) BRITTON TOXICITY IN AN ADULT GOAT

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Seasonal outbreaks of respiratory disease, presumably due to perilla mint (*Perilla frutescens* (L.) Britton), have been reported across the southern United States. This is the first report of perilla mint toxicity in Oregon and provides the first detailed description of the associated histopathology in a small ruminant. Autopsy of a four year-old LaMancha doe submitted after an unexpected death revealed a significant interstitial pneumonia characterized by non-collapsed lungs with diffuse pulmonary edema, and thickened interlobular septae. Histologically, there was marked alveolar and interlobular edema with rare hyaline membranes. Unlike reports described in cattle, marked proliferation of Type II pneumocytes was not observed histologically. All ancillary diagnostics which included a PCR respiratory panel (IBR1, BVDV, BRSV, and PI3), aerobic and mycoplasma culture, yielded negative results. During subsequent discussions, the owner reported the goat had grazing access to perilla mint which guided a putative diagnosis of perilla mint toxicity.

SP-66: RETROSPECTIVE STUDY OF FATAL PNEUMONIA IN BACKYARD HORSES OF CALIFORNIA

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The purpose of this retrospective study was to investigate the ways in which fatal pneumonia impacts backyard horses in California and to understand the role that diseases in other organs play in the development of pulmonary lesions. One hundred and fifty-six backyard horses that died with pulmonary lesions submitted for autopsy to the California Animal Health and Food Safety Laboratory System from January 2015 to June 2020 were available for this study. The most common finding was bronchopneumonia (35%), followed by interstitial pneumonia (29%), embolic pneumonia (21%), granulomatous pneumonia (13%) and pleuritis (2%). Animals were divided in two major groups: Animals that died or were euthanized due to pulmonary causes (Group 1, n=74), and animals that died or were euthanized due to non-pulmonary causes but had lung lesions present at the time of autopsy (Group 2, n=82). In group 1, the most commonly isolated bacteria in cases of bronchopneumonia were *Streptococcus equi* subsp. *zooepidemicus* (48.5%), *Klebsiella pneumoniae* (12.1%), and *Actinobacillus equuli* subsp. *haemolyticus* (9.1%). In group 2, the most significant extrapulmonary lesions responsible for death were found in the gastrointestinal tract (30%), multiple

systems (septicemia/toxemia; 27%), musculoskeletal system (12%), nervous system, and cardiovascular system. Of horses that died or were euthanized due to a primary gastrointestinal tract problem, 48% had lung lesions that were considered secondary to the gastrointestinal disease, which frequently had an infectious cause. This study illustrates the way pneumonia impacts backyard horses and provides perspective on pulmonary involvement when animals die of non-pulmonary causes.

SP-67: SEVERE UNILATERAL CARBONATE-CALCIUM NEPHROLITHIASIS IN A HORSE

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Urolithiasis is an uncommon disorder reported in horses with the urinary bladder and urethra being the most common affected anatomical locations. Calculi located in the kidneys and ureters are often underdiagnosed because they are rarely associated with clinical signs and when signs are present they are often nonspecific: weight loss, anorexia, hematuria, dysuria, colic, and polyuria/polydipsia. A 26-year-old, Thoroughbred gelding presented for an acute episode of colic of 5 hours duration. The primary clinical sign was lateral recumbency. The horse had a 10-year history of intermittent mild colic that resolved with a single oral dose of flunixin meglumine. On abdominal palpation per rectum, tight bands and marked distension of the large colon were present, precluding a complete evaluation of the abdomen. Hematologic analysis revealed an elevated creatine kinase of 665 U/L (reference range: 120-470 IU/L) and mildly increased creatinine of 2.3 mg/dL (reference range: 0.6-2.2 mg/dL). Abdominocentesis yielded serosanguineous fluid with a lactate of 9.6 mmol/L (<2 mmol/L). Due to the severity of the clinical signs, exploratory laparotomy was recommended. Surgical intervention was declined and the horse was euthanized. Limited postmortem evaluation was performed. A 3.633 kg nephrolith measuring 23.6 x 16.2 x 8.9 cm was completely effacing the left kidney. The calculus was submitted for analysis via optical crystallography and infrared spectroscopy (ATR and FTIR). The analysis revealed a 100% calcium-carbonate nephrolith. Carbonate calcium calculi are the most common subtype in horses. Nephrolithiasis should be considered as a differential diagnosis for colic and hematologic evidence of azotemia in horses.

SP-68: FARM ANIMAL FORENSIC INVESTIGATION: BAA-KING UP THE WRONG TREE?

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The carcasses of two neonatal lambs from different litters were found with signs of trauma at a farm. The farmer reported that the lambs had been otherwise healthy, and that eight more lambs had previously gone missing. Although post-mortem examination found no signs of pre-existing conditions to explain their sudden demise, evidence of severe, acute thoracic trauma with rib fractures, extensive internal lacerations, and internal haemorrhages were identified in both lambs. Furthermore, one had a depressed

skull fracture. After careful consideration of the post-mortem findings, combined with information from the farmer and veterinarian, the cause of the deaths of these two lambs and the disappearance of the other eight were ultimately suspected to be associated with husbandry- and environment-related factors. A sudden drop in ambient temperature was hypothesised to have led to lambs huddling too close to adults and becoming accidentally crushed beneath them, resulting in traumatic injuries. Predatory bird activity, immediately before death, could also have contributed to the lesions. Additional information from the veterinarian also highlighted that the eight missing lambs were thought to be weak and so were likely taken by foxes. This case report shows the importance of keeping an open mind in forensic pathology. It is essential that cases are considered objectively, to determine the reality of the situation and ensure that pre-determined assumptions do not skew the outcome.

SP-69: THE HONEYBEE CYTOLOGY ATLAS : A RESOURCE FOR VETERINARIANS

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Despite being a common production animal and one of the most important pollinators on Earth, veterinarians are not as involved in bee diagnosis and research as they are in mammalian species. Veterinary pathology is at the basis of diagnosis and research in most animal species, but it is only at its infancy in bees. Although knowledge is increasing in bee histopathology, cytology is still underexploited. Indeed, cytology is a fast, simple and cost-effective procedure, but has yet to be standardized for honeybees (*Apis mellifera*). The aim of this study is to identify and characterize bee cells in order to create a comparative cytology-histology atlas for normal bee tissues to help veterinarians and researchers improve and maintain bee colony health. After manual removal of the bee's exoskeleton, body parts (head, thorax, abdomen) or individual organs were used to produce touch imprints before being fixed and processed to histology slides. Using methanolic Romanowsky stain coloration (modified Giemsa), microscopic evaluation of cytology smears revealed a wide range of cells types which were then compared to the corresponding histology slides. Our results will provide a database of normal tissues for future research and diagnostic needs. A better understanding of normal bee cytology will help veterinarians and researchers recognize abnormal or diseased cells and will therefore contribute to better bee colony management.

SP-70: ESTABLISHMENT OF A METHYLATION-SPECIFIC QPCR TO ASSESS CPG ISLAND METHYLATOR PHENOTYPE IN SPONTANEOUS COLORECTAL CARCINOMA OF RHESUS MACAQUES (MACACA MULATTA)

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Background: Colorectal carcinoma (CRC) is the second leading cause of cancer-related death in humans and the most prevalent malignancy in rhesus macaques. A subset of human CRCs exhibit aberrant DNA methylation of tumor suppressor gene promoters, demonstrating the so-called CpG island methylator phenotype (CIMP). No information is available about this epigenetic phenotype in rhesus. Rhesus are an unrivaled translational model of spontaneous diseases, and thorough molecular characterization of these neoplasms is imperative to establish this model. Objective: We aimed to develop a novel qPCR assay to detect dysmethylated rhesus DNA promoters and deploy this assay on biobanked CRCs (FFPE) to determine CIMP status. Methods: We designed rhesus-specific primers and methylation-specific qPCR probes for bisulfite converted genomic DNA to investigate the methylation status of the 5'-UTR CpG islands of *MLH1*, *CACNA1G*, *CDKN2A*, *CRABP1*, and *NEUROG1*. Methylation-independent primers and probes targeting *ACTB* were used as a reference. To validate this method, we spiked untreated peripheral leukocyte DNA with artificially hypermethylated DNA and prepared serial dilutions. Results: Our qPCR selectively detected artificially hypermethylated DNA, as demonstrated by consistently lower Ct values for methylated samples compared to untreated samples. In serial dilutions, methylated DNA could be detected in a quantitative manner. Conclusion: We have established a qPCR assay for the detection of CIMP status in naturally-occurring CRCs of rhesus macaques. This work and further molecular phenotyping contributes to the development of an exceptional preclinical model. From a veterinary perspective, this work may contribute to a "liquid biopsy" approach for early detection of rhesus CRC.

SP-71: CATEGORIZATION OF NEONATAL KITTEN DEATHS: A RETROSPECTIVE STUDY OF 62 CASES (1999-2020)

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Few attempts have been made to examine the causes of neonatal kitten deaths in the United States. In this report, we retrospectively searched the University of California, Davis Veterinary Medicine Teaching Hospital pathology database to identify and categorize causes of deaths in neonatal kittens, defined as 0 to 28 day-olds, in a 21-year period (1999-2020). Our search parameters resulted in 62 autopsy reports. The most common cause of death was infectious (45/62) followed by congenital (13/62). Other less common causes were toxic (2/62), trauma (2/62), unknown (2/62), metabolic (1/62), and other (1/62). Four cases fell under 2 categories, infectious and congenital, and in 1/4, infection was associated with the congenital malformation. Of infections, lung (23/45) and intestinal (9/45) origins were most common. Commonly cultured etiologies for bacterial infection regardless of affected site included E. coli (8), Streptococcus sp. (7), and Pasteurella multocida (3). Clostridium piliforme infection was suspected in 3 cases through silver staining that affected the ileocecocolic junction and liver. Common viral etiologies for respiratory disease included feline herpesvirus-1 (8) and feline calicivirus (2). Aspiration pneumonia was suspected in 5 cases. Feline parvovirus was only suspected in 1 case. Common non-infectious causes of death included

palatoschisis (3/62), CNS malformation (3/62), cardiac disease (2/62), and head trauma (2/62). This study suggests that infection, mostly bacterial followed by viral, is a major cause of death in neonatal kittens. These results can give insight into proper care and screening exams of our neonatal kittens for caregivers.

SP-72: A CASE OF FELINE NEUROTROPIC LYMPHOMA

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Lymphosarcoma is the most common malignant neoplastic disease in cats. It originates in naturally occuring lymphoid tissue and can metastasize to different sites. It tends to manifest as multicentric, thymic or intestinal forms. Uncommonly, neoplastic lymphocytes invade nervous structures such as nerves. Neurotropic lymphoma, also called neurolymphomatosis, has been described in bovine, avian, canine and feline species. This case describes the presentation of neurotropic lymphoma in a domestic cat. A ten-year-old, neutered male, domestic shorthair feline had a month-long history of acute progressive right rear limb paresis. The patient had not been using or placing the right rear limb. There was no reported history of trauma or injury. Upon physical examination, significant muscle atrophy was noted in the affected limb as well as a lack of conscious proprioception and superficial pain response. Because of the marked progression, the affected limb was amputated. Nothing of relevance was found during the gross examination. Sections of muscle, bone, and synovial membrane were collected. Histopathology revealed moderate muscle atrophy and a mild lymphocytic infiltrate in the nerve attached to the muscle. Extra slides from the sciatic nerve were added and marked lymphocytic infiltration of the nerve was observed. The axons were compressed and disrupted by the lymphocytic population. This lymphocytic population found in the nerves is compatible with peripheral nerve neurolymphomatosis or neurotropic lymphosarcoma. CD-3 positive immunohistochemistry markers revealed that the neoplastic cells were T-cells. Unfortunately the cat was euthanized two weeks after the diagnosis due to paresis on the other limb.

SP-73: DIAGNOSTIC AND PROGNOSTIC MARKERS OF CANINE PERIPHERAL NERVE SHEATH TUMORS

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Peripheral nerve sheath tumors (PNSTs) arise from supporting cells of nerves and are subjectively classified as benign (BPNST) or malignant(MPNST). However, there are overlapping histologic features of BPNSTs and MPNSTs, making a definitive diagnosis challenging. Distinction between BPNSTs and MPNSTs is vital as MPNSTs are aggressive neoplasms that pose therapeutic challenges and have the potential to metastasize. Explicit histologic and immunohistochemical characteristics defining BPNSTs versus MPNSTs are currently lacking. By examining the histologic,

immunohistochemical, and clinical outcome profiles of 40 BPNSTs and 40 MPNSTs we have established diagnostic criteria for these ambiguously diagnosed tumors. Histologic evaluation included mitotic count, presence of necrosis, and local invasion. MPNSTs exhibited a significantly higher mitotic count than BPNSTs ($p<0.0001$). Additionally, significantly more MPNSTs had necrosis ($p<0.05$) and invasion into surrounding tissue ($p<0.0001$) than did BPNSTs. Patient population data indicated that the three most common anatomic locations of PNSTs included the head/neck region, proximal forelimb, and spinal canal, and the breeds most commonly diagnosed were Labrador and Golden Retrievers. Lastly, clinical outcome data indicated that age at diagnosis ($p<0.05$) and age at death ($p=0.0005$) were both significantly younger in patients with MPNSTs than those with BPNSTs. There was no significant difference in mean survival times between patients with BPNSTs and MPNSTs. In this study, we establish histologic and immunohistochemical criteria for malignancy in PNSTs to define diagnostic and prognostic criteria for classifying PNSTs in dogs.

SP-74: WHITE CHICK SYNDROME IN COMMERCIAL BROILERS IN TEXAS

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Eleven, one day-old chicks presented to the Texas A&M Veterinary Diagnostic Laboratory for necropsy. Submitted with these chicks were two jars of formalin fixed livers from chicks sampled at the hatchery. The chicks originated from a hatchery with a complaint of decreased hatchability of nearly 20% occurring in the eggs set from one breeder flock. Six chicks had pale, white down and were notably smaller in size than the other five, which had more normal yellow down. On necropsy, the pale chicks had livers with slight green-brown hues and two of the chicks had yolks sacs with green-yellow contents. Livers in one of the jars had green discoloration more prominent than the seen in the birds submitted for necropsy. Histologically, there was severe hyperplasia of bile ducts with heterophilic to necrotizing cholangiohepatitis and periportal fibrosis. Extramedullary granulopoiesis was seen in the liver and kidneys. In the kidneys, tubules were multifocally dilated and filled with eosinophilic debris. Virus isolation and PCR of liver and intestines conducted at the Mississippi Poultry Research and Diagnostic Laboratory confirmed the presence of chicken astrovirus. Overall, the pathologic changes in this case were characteristic of white chick syndrome caused by chicken astrovirus. Astroviruses are relatively ubiquitous in the environment and it is thought that white chick syndrome is caused by vertical transmission of the virus to chicks from naïve breeder hens exposed for the first time after the start of lay. Hatchability in the affected flock recovered to 87% within two weeks after the initial decline.

SP-75: THE EFFECT OF EQUINE (EQUUS CABALLUS) SUPPORTING LIMB LAMINITIS ON HOOF LAMELLAR INTERLEUKIN-17 AND CD3 EXPRESSION

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Supporting limb laminitis (SLL) is a debilitating and often fatal complication of severe lameness. Altered limb loading and decreased tissue perfusion compromise hoof lamellae, resulting in failure of digital suspension within the hoof capsule. Our previous study showed activation of the interleukin (IL)-17 pathway, the major pro-inflammatory pathway in human psoriasis, in SLL. IL-17, the pathway's effector cytokine, is produced primarily by Th17 cells (a subset of CD3+ T cells) and other cell types. We have previously shown CD3+ T cell infiltration of lamellae correlates with severity of SLL, but the IL-17 status of these cells was unknown. Here, we identified CD3+ and IL-17+ cells by dual indirect immunofluorescence in severe SLL cases (N=15) and controls (N=8) and determined if IL-17 expression localized to CD3+ T cells using epifluorescent microscopy and ImageJ and QuPath analysis. Initial results from 9 severe cases and 8 controls suggest that severe SLL may be associated with increased numbers of CD3+, IL-17+, and CD3+/IL-17+ cells relative to controls. However, not all IL-17+ cells co-expressed CD3 as expected. We observed IL-17 expression by other cell types, such as keratinocytes and uncharacterized CD3- leukocytes, suggesting a possible role for these cells in IL-17 pathway activation. These data improve our understanding of the pathogenesis of SLL and some similarities with human psoriasis, suggesting new approaches for improved diagnosis, treatment, and prevention of SLL.

SP-76: PATHOLOGIC CARDIOVASCULAR TISSUE ALTERATIONS ASSOCIATED WITH EXPERIMENTAL ANTI-FIBROTIC THERAPIES

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Current therapeutic options for diseases associated with fibroplasia, such as liver cirrhosis and idiopathic pulmonary fibrosis, are very limited. Tadalafil, a phosphodiesterase 5 inhibitor commonly prescribed for erectile dysfunction, which has anti-fibrotic properties, and the investigational anti-fibrotic drug IPW-5371 were examined for therapeutic fibrosis therapy in an irradiated mouse model. The combination of agents unexpectedly resulted in increased mortality in C57 B6/N male mice, but not in female mice. This finding indicates that there is very little information regarding the possible relative contraindications between these two drugs in both mouse and human models. Death resulted from hemorrhage into body cavities, and this study aimed to evaluate the pathologic findings relating to these deaths. We investigated the effects of tadalafil and IPW-5371 administration on the cardiac and left ventricular outflow tract tissue in 134 irradiated C57 B6/N male and female mice. We hypothesized an increase in inflammation, hemorrhage, degeneration, as well as various other lesions would be increased in mice that had the combination treatment compared to other mice. A total of 61, 6-month-old male mice were statistically analyzed. Mice that were administered drugs had significantly higher frequencies of tissue changes or alterations than mice that were not administered drugs. Mice that were given IPW-5371 either alone or in combination with tadalafil had higher frequencies of lesion scores than mice that were not administered IPW-5371. These

findings indicate that subclinical tissue alterations exist in mice administered this drug combination at a greater frequency than mice that were not administered drugs.

SP-77: EFFECTS OF RECEPTOR TYROSINE KINASE INHIBITION ON CANINE GLIOMA CELL LINES IN VITRO

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Canine gliomas are the second most common primary brain tumor in dogs. With no existing standard-of-care, they present a clinical challenge to treat. Recent work reveals that mutation and genomic amplification of receptor tyrosine kinases (RTKs) are common events driving tumor formation in dogs, particularly platelet derived growth factor alpha (PDGFRA). Toceranib phosphate (Palladia®) is a tyrosine kinase inhibitor that blocks autophosphorylation of RTKs and preferentially inactivates PDGFRA. We hypothesize that PDGFRA activity drives proliferation in a subset of canine glioma cells, and treatment with toceranib inhibits cell growth. In this study, we determine IC50 dose-response curves for toceranib phosphate in three canine glioma cell lines, characterize expression and signal transduction of several RTKs in canine glioma cell lines, and characterize the effect of toceranib on canine glioma cell growth and RTK activity through downstream RTK pathways. Dose-response curves were determined using propidium iodide cell count and viability flow cytometry assays, RTK expression and activity was visualized via Western blot and immunocytochemistry, and cytotoxic and cytopathic effects were evaluated by flow cytometry via Annexin V and cell cycle analysis assays. Each patient-derived canine glioma cell line exhibited RTK activity, including PDGFRA, and was susceptible to inhibition by toceranib. Relative expression of RTKs and downstream target proteins varied by cell line, as did the magnitude of inhibition. The MAP kinase pathway appears to be an important pathway utilized across cell lines and inhibited by toceranib. This highlights a potential adjuvant therapy for canine glioma patients more accessible than surgery and radiation.

SP-78: OPTIMIZING THE DETECTION OF PERSISTENT AMDOPARVOVIRUS INFECTIONS IN ZOO-HOUSED RED PANDAS (AILURUS FULGENS)

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Persistent (life-long) Amdoparvovirus (APV) infections are common in many carnivore species. We have established that 50% of US zoo-housed red pandas (*Ailurus fulgens*) are infected with the recently discovered Red Panda Amdoparvovirus (RPAV). Host-specific APVs can be pathogenic in other species, so the high prevalence of RPAV in this endangered species is concerning. We hypothesize that RPAV causes persistent infections that can be associated with disease. An accurate, sensitive, and reliable method of detection is needed to understand the population-level health impact of RPAV in red pandas. We developed and validated a SYBR Green-based quantitative PCR (qPCR) assay targeting a 121-nt-long fragment of the viral capsid gene. Initial applications of this assay have so far supported two studies. The first study used a single cohort of infected animals in a prospective analysis of persistent infection and

shedding. A 5-year longitudinal fecal shedding survey demonstrated consistent viral shedding from two clinically normal animals for the duration of the study period. We also demonstrated fecal shedding from two previously RPAV-negative red pandas after they were introduced to an infected cohort. The second study is a retrospective analysis of RPAV-associated disease. In this study, qPCR is used to detect RPAV infections in archived red panda necropsy tissues and followed with cell and tissue distribution studies by *in situ* hybridization (ISH). Combined results demonstrate RPAV is present in putative sites of asymptomatic persistence (spleen, intestine) and in association with significant lesions including myocarditis, tubulointerstitial nephritis, pharyngitis, and interstitial pneumonia.

SP-79: LUNG HISTOPATHOLGY OF NEUTROPHIL DEPELETED MICE IN A PNEUMOCOCCAL PNEUMONIA MODEL

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Background *Streptococcus pneumoniae (Sp)* is a leading cause of fatal pneumonia worldwide. Current commercial vaccines have limited serotype coverage. Monoclonal antibodies (mAbs) targeting conserved surface antigens of *Sp* are protective, including those targeting the pneumococcal histidine triad protein (PhtD). However, the immune pathways in which PhtD mAbs function are unknown. Previously, we found complement depleted mice had significantly lower survival than mice treated with PhtD mAbs, indicating complement involvement in anti-PhtD mAb efficacy. **Objective** The goal of the current study was to evaluate the therapeutic efficacy of PhtD mAbs in neutrophil depleted mice in a pneumococcal pneumonia model and evaluate corresponding lung histopathology. **Methods** C57BL/6 mice were neutrophil depleted by administration of RB6-8C5(Ly6G/Ly6C) antibody and treated with the mAb PhtD3, while control counterparts received a non-specific mouse isotype control antibody. Remaining control groups received PhtD3 mAb alone or isotype control antibody. All mice were challenged intranasally with a pneumococcal serotype 3 strain (WU2) and monitored for clinical signs. Lungs from three mice in each group were stained with H&E and Gram stain. Histological sections were assessed for presence of pneumonia, bacterial invasion, and degree of inflammation. **Results** Neutrophil depletion did not significantly affect the survival of mice treated with the mAb PhtD3. Upon histological analysis, neutrophils were present in all groups, indicating incomplete neutrophil depletion. Histological sections are currently undergoing scoring and review. **Conclusions** Due to incomplete neutrophil depletion, conclusions regarding neutrophil involvement in PhtD3 function cannot be made at this time. Further studies are warranted to optimize neutrophil depletion.

SP-80: RESPIRATORY DISEASE IN A RABBIT (ORYCTOLAGUS CUNICULUS)

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A 6-year-old, castrated male, Dutch rabbit (Oryctolagus cuniculus) presented for an acute onset of dyspnea. Initial examination and diagnostics revealed abnormal

respiratory sounds and a suspected mass in the cranial lungs. Culture and sensitivity of a swab from the nasal cavity resulted in Staphylococcus spp. Clinical signs progressed for 4 months despite antibiotic therapy, and ultimately the patient died under sedation while being re-examined. Post-mortem examination revealed abscesses of the left cranial lung and fibrinous adhesions between left lung and the thoracic wall. The remaining lung lobes were diffusely dark red. Microscopic findings revealed pulmonary abscesses with alveolar type II pneumocyte hyperplasia and heterophilic and histiocytic alveolitis. Upper respiratory pathology included moderate heterophilic and lymphoplasmacytic rhinitis and tracheitis with segmental cilia loss. Aerobic/anaerobic culture of the pulmonary abscesses did not result in any bacteria being identified. Likewise, Gram and PAS stains did not highlight any organisms in lung tissue. The pathological findings are consistent with a bacterial infection. Four common bacterial pathogens cause respiratory disease in rabbits; these are: Pasteurella multocida, Staphylococcus aureus, Bordetella bronchiseptica and Klebsiella pneumoniae. Given the previous culture results prior to antibiotic administration, and appearance of lesions, Staphylococcus aureus is considered the most probable cause of chronic respiratory disease in this case. The absence of bacterial isolates is likely due to the prolonged treatment of antibiotic therapy. This diagnostic impediment is not uncommon and should be considered clinically before initiating therapy and when rendering a definitive diagnosis based on ancillary test results.

SP-81: EXTRAMEDULLARY HEMATOPOIESIS IN THE CEREBROSPINAL FLUID OF A YORKSHIRE TERRIER

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Case Report A 14-year-old, spayed female, Yorkshire Terrier, was presented to the University of Florida for evaluation of intermittent chronic right hind limb lameness. Physical exam findings included atrophy and proprioceptive deficits of the right hind limb with absent patellar and withdrawal reflexes. CBC revealed a mild thrombocytosis (467 K/ml, RI 134 – 396). CT imaging showed an extensive intradural, extramedullary mass, with extension to the right L5-L7 nerve roots. A lumbar cerebrospinal fluid tap revealed a mild mixed cell pleocytosis (TNCC/µL: 33, RI <5) with non-degenerate neutrophils (47%), small lymphocytes (45%), large mononuclear cells (6%), and eosinophils (1%) with nucleated erythrocytes (30nRBCs/100WBCs). Increased numbers of metarubricytes and polychromatophilic rubricytes were noted with lesser basophilic rubricytes and rubriblasts with no evidence of polychromatophilic erythrocytes. Rare myeloid precursors were identified. A right hemipelvectomy and hemilaminectomy was performed and the mass was submitted for histopathologic evaluation. A presumptive diagnosis of malignant peripheral nerve sheath tumor was made, but with the large number of multinucleated giant cells, an anaplastic sarcoma could not be excluded. **Conclusions** Although uncommon, the presence of hematopoietic precursors in cerebrospinal fluid can be associated with extramedullary hematopoiesis in the spinal and cranial dura mater along with bone marrow contamination as reported in both

humans and dogs. Extradural hematopoiesis may be secondary to hypoxia, local ischemia, leakage from bone marrow or secondary to a neoplastic process. This case demonstrates an example of erythroid and lesser myeloid extramedullary hematopoiesis, likely secondary to an infiltrating neoplasm and altered local cytokine environment.

SP-82: SPECIATION OF THE ETIOLOGIC AGENT OF CANINE LEPROID GRANULOMA SYNDROME USING PCR ON FFPE TISSUE SAMPLES

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Canine leproid granuloma syndrome (CLGS) has a widely recognized, classic presentation and has been documented around the world. The etiologic agent inciting the condition has yet to be determined beyond suggestion of it belonging to the *Mycobacterium* genus. This study seeks to confirm that the CLGS agent is a novel *Mycobacterium* species and to characterize it by phylogenetic analyses. Eight previously published PCR primer pairs targeting various regions of the bacterial genome were evaluated and amplicon sequences were compared with those cataloged in the NCBI BLAST database. One pair of PCR primers targeting the mycobacterial ITS1 region generated high quality amplicon sequences that allowed for correct identification of agents from several known *Mycobacterium* diseases. PCR using these primers was performed on 35 samples from 26 CLGS cases diagnosed by histology, and 10 cases generated bands of sufficient quality to be sequenced. 8 case sequences exhibited 100% homology and one additional case sequence exhibited >99.75% homology with a published but unnamed sequence from a previous CLGS study. *M. simiae* was the closest (>96%) established species to this CLGS sequence and these 9 case sequences. These results confirm that the CLGS agent is a member of the *Mycobacterium* genus and strongly suggest that this CLGS agent represents a novel, globally distributed species. Further study will include shotgun sequencing of selected cases to assemble the whole genome of this potentially novel pathogen.

SP-83: NRP2 SIGNALING IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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Background: Malignant peripheral nerve sheath tumors (MPNST) are soft tissue sarcomas that have a notoriously poor response to conventional chemotherapy/radiation. Signaling pathways that contribute to MPNST pathogenesis are not yet fully defined. Neuropilins are cell surface receptors that function as co-receptors for various transmembrane receptors and interact with many ligands. One such ligand is semaphorin 3F (SEMA3F), which is an extracellular signaling protein that plays a role in many pathways of growth and development, including tumorigenesis. **Objective:** Our objective was to determine how NRP2 and SEMA3F modify MPNST phenotypes. Using human MPNST cell lines, we investigated the role for the neuropilin receptor NPR2 and its associated ligand SEMA3F in MPNST growth and migration.

Methods: MPNST cells underwent a co-culture migration assay with 293TN cells expressing SEMA3F. MPNST cells with NRP2 knockdown were analyzed for rate of cell growth by serial Cyquant assays, migration by transwell migration, and proliferation and apoptosis by caspase 3/7 assay. **Results:** Migration of MPNST cells was increased in the presence of SEMA3F. Cyquant assays revealed NRP2 knockdown cell lines have a lower rate of growth compared to controls. NRP2 knockdown cells lines also exhibited increased apoptosis compared to control lines. **Conclusions:** Our data suggest that NRP2 knockdown cell lines undergo reduced proliferation and increased apoptosis compared to controls, suggesting that NRP2 is important for MPNST cell growth. Our data also show that MPNST migrated at increased rates in the presence of SEMA3F, suggesting that NRP2-SEMA3F interaction may regulate MPNST behavior.

SP-84: MYCOBACTERIUM ULCERANS ECOVAR LIFLANDII IN PET DWARF AFRICAN CLAWED FROGS (HYMENOCHIRUS SPP.)

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Mycobacterium ulcerans ecovar *liflandii* is a nontuberculosis mycobacterium (NTM) responsible for mortality in research African Clawed Frog *(Xenopus tropicalis)* colonies in the United States and Europe. It is a variant of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer disease in humans. The zoonotic potential of *M. liflandii* is unknown. During 2019 to present, 9 African Dwarf frogs (ADF; *Hymenochirus spp.*) submitted by pet owners to the Washington Animal Disease Diagnostic Laboratory (WADDL) for evaluation of cutaneous ulcerative lesions were diagnosed with mycobacteriosis. Animals originated from the states of WA, NY, CA, MA, CT, VT, and OR. Histologic lesions consisted of a necrotizing and ulcerative dermatitis with myriad Fite's acid-fast positive bacilli. Necrotizing lesions were also observed in adipose tissue, serosal surfaces of viscera, liver, and kidney. Mycobacterial 16s-23s ITS PCR and DNA sequencing from paraffin-embedded tissue most closely matched (greater than 99% sequence identity) that of the *Mycobacterium marinum-M. ulcerans* complex. To distinguish between *M. marinum*, *M. ulcerans* and *M. ulcerans liflandii,* variable number tandem repeat (VNTR) typing of the enoyl reductase domain on the mycolactone plasmid was performed. All were identified as *M. liflandii.* This is the first known report of *M. liflandii* infections occurring in ADF. ADF are a common species in the pet trade and the presence of *M. liflandii* in household fish tanks suggests a wider environmental prevalence of the bacterium than previously thought. Additional studies are needed to determine if *M. liflandii* is of public health importance.

SP-85: CANINE OSTEOSARCOMA CELLS INDUCE TNFa AND CXCL10 mRNA EXPRESSION IN MACROPHAGES

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Macrophages are innate immune cells that adopt a wide variety of phenotypes, the extremes of which are pro-inflammatory (M1) or anti-inflammatory (M2). Tumors

reprogram macrophages directly or through the tumor microenvironment (TME) to induce an immunosuppressive phenotype permissive to tumor survival and growth. We hypothesized that canine osteosarcoma cells would induce the anti-inflammatory M2 macrophage phenotype. Our aims were to 1) determine whether canine osteosarcoma cells secrete signals that alter gene expression in canine macrophages, and 2) determine whether pre-stimulatation of osteosarcoma cells with interferon-gamma (IFNg), a cytokine in the TME, would alter this effect. Abrams osteosarcoma cells were cultured in media for 24 hours to generate conditioned media (Abrams-CM). Canine macrophage-like DH82 cells were incubated with filtered Abrams-CM for 24 hours. Relative expression of macrophage M1 genes (TNFa, CXCL10) and M2 genes (TGM2, CD23) was measured by quantitative PCR (qPCR). We found that Abrams-CM significantly upregulated macrophage expression of TNFa and CXCL10, but had no effect on TGM2 or CD23. Compared to Abrams-CM, CM from IFNg-treated Abrams cells had no additional effect on macrophage expression of TNFa or CD23. In stark contrast, CM from IFNg-treated Abrams cells induced a 1000-fold increase in CXCL10 and 5-fold increase in TGM2. Together, these data show that canine osteosarcoma cells induce a pro-inflammatory, rather than an anti-inflammatory phenotype in macrophages, and that pre-stimulation with IFNg dramatically alters this effect. These studies begin to tease apart the complex signaling between osteosarcoma cells and macrophages, with potential implications for the treatment of canine osteosarcoma.

: ABDOMINAL MESOTHELIOMA WITH PULMONARY METASTASIS IN A COTON DE TULEAR DOG

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The emaciated body of an eight-year-old castrated male Coton De Tulear was presented to the Veterinary Diagnostic Laboratory at the University of Illinois for postmortem examination after a 1-month history of decreased appetite, weight loss, and diarrhea. Diagnostics prior to submission identified mild abdominal effusion, a radiographic interstitial pulmonary pattern, and worsening azotemia. Patient was euthanized at the clinic due to progressive condition. Postmortem gross examination revealed marked and near diffuse plaque-like thickening of the visceral and parietal peritoneum; the parietal peritoneum and visceral peritoneum of the kidneys, intestines, and urinary bladder were most severely affected. Similar thickening of the lung pleura was identified. Routine histopathologic examination identified stellate to spindloid neoplastic cells arranged in nests and irregular trabeculae that frequently infiltrated the underlying tissue. Neoplastic cells had variably distinct cell margins, scant to moderate eosinophilic cytoplasm, and a round to oval nucleus containing coarse to vesiculate chromatin and one to several variably sized magenta nucleoli. Marked anisocytosis, anisokaryosis, and frequent mitoses were observed in the neoplastic cell population. Neoplastic cells had cytoplasmic reactivity to cytokeratin immunohistochemical staining, in addition to cytoplasmic reactivity to vimentin immunohistochemical staining. This

reactivity and the observed morphology and cell distribution confirmed a diagnosis of the solid form of abdominal mesothelioma with pulmonary metastasis.