



2024 ACVP/ASVCP Annual Meeting Accepted Late-Breaking Abstracts

1: LAWSONIA INTRACELLULARIS INFECTION IN A CAPTIVE RED PANDA (AILURUS FULGENS)

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Lawsonia intracellllularis is a well-known cause of proliferative and necrohemorrhagic enteropathies in domestic and exotic animals. A captive red panda, four months after giving birth was found lethargic, anorexic and with hunched back, signs suggestive of abdominal discomfort. Three days later it was found dead and postmortem examination was performed. On gross examination, throughout the intestinal tract, most severe in ileum and colon, the mucosa was thickened, rugose, and covered with a fibrinonecrotic membrane and hemorrhage. Histologically, large gram-negative bacteria colonies, hemorrhage, fibrin and degenerate leukocytes obscured markedly hyperplastic colonic crypts that sometimes invaginated into the submucosa. Silver staining revealed numerous intracolonic, short curved rods that had marked, diffuse, positive reactivity with antibodies against *Lawsonia intracellularis*. In pigs, chronic proliferative enteropathy can suddenly become an acute clinical case of necrotizing enteritis if there is colonization of the intestine by pathogenic gram-negative bacteria (e.g. *E. coli*, *Salmonella*, *Klebsiella*). The chronic changes in the intestinal mucosa associated to *L. intracellularis* likely predispose to this condition. Similarly, in this case, besides *L. intracellularis*, there was a second population of extracellular gram-negative bacteria associated to areas of necrosis and thrombosis. These bacteria could have facilitated or triggered an acute onset of gastrointestinal signs in this animal. The role of *L. intracellularis* as a pathogen in zoo species is not well understood. There are historical reports of necrotizing and hemorrhagic colitis in red pandas however, it is unclear if *L. intracellularis* was involved in those cases.

2: DIAGNOSIS AND SURGICAL REMOVAL OF A CORNEAL DERMOID IN A RED-EYED TREE FROG, AGALYCHNIS CALLIDRYAS (ANURA, HYLIDAE), FROM THE TENORIO VOLCANO, COSTA RICA

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A male, red-eyed frog (*Agalychnis callidryas*) was collected from the Tenorio Volcano region in Costa Rica due to the presence of a unilateral ocular abnormality affecting its left eye. Approximately 70% of the cornea was replaced by a well demarcated, 5.0 mm in diameter, opaque, bright green and roughened tissue compatible with skin. The affected eye was enucleated under established ophthalmic surgical procedures. A dermoid was confirmed by histopathology based on the finding of amphibian cutaneous (epidermal and dermal) tissue replacing the cornea and characterized by the presence of keratinized epidermis, spongy dermis, and integumentary glands. After a successful post-operative recovery period the frog was released back to the wild, demonstrating the viability of medical intervention and surgery in wild amphibians. Dermoids are congenital lesions that result from a failure in the differentiation of the superficial ectoderm to generate normal corneal epithelium and

the subsequent development of epidermis and dermal tissues in the area. Corneal dermoids have been reported in horses, cattle, sheep, wildebeest, pigs, rats, dogs, cats, birds, and humans, this being the first case reported in amphibians. In the locality where the frog was found, several other ophthalmic abnormalities have been documented in other amphibians. Active monitoring of wild amphibians is ongoing, and possible causes of ophthalmic abnormalities in the area are being investigated.

3: IDENTIFICATION OF FRANCISELLA TULARENSIS INFECTION AND CLINICAL TULAREMIA IN STRANDED BELUGA WHALES IN ALASKA

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Background: Cook Inlet beluga whales (CIBs) are critically endangered and reside year-round in the waters adjacent to Anchorage, Alaska. In 2023, 7 CIBs stranded within one month; a similar event with ten animals occurred in 2017. Several of these belugas demonstrated pleuritis, multifocal bronchopneumonia, multifocal random hepatitis, and lymphadenitis.

Objectives: Determine the cause of pathology of these beluga whales using updated molecular techniques.

Methods: Total nucleic acid from pooled lung, lymph node and blowhole swab sample from a 2023 whale was agnostically amplified and analyzed by rapid metagenomics using a nanopore sequencing device. After detection of a signal for *F. tularensis*, real-time PCR was performed on two whales from 2023 and one from 2017. Further characterization to type was performed by PCR and amplicon-based multilocus sequence typing (MLST).

Results: By metagenomics, approximately 1180 reads (N50=250nt, average Q=9) mapped across the 1.9Mbp *F. tularensis* genome in addition to matching 16S rRNA reads ($n=200$). In two 2023 whales tested, both liver and lung and in one 2017 whale, mediastinal lymph node were positive by PCR for *F. tularensis*. By MLST, in over 4107 bp comprising the *fabH*, *tpiA*, *sdhA*, *rpoA*, *pgm*, and *groEL* genes, the sequences were identical to a harbor seal case that occurred in Washington in 2023, as well as other *F. tularensis* Type B isolates from the northwestern USA.

Conclusion: We report three cases of tularemia due to *F. tularensis* type B in three stranded CIBs. This is the first report of tularemia in a cetacean.

4: ASPERGILLUS OCHRACEUS MENINGOENCEPHALITIS AND PYELONEPHRITIS IN A DOG

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Aspergillus spp. are ubiquitous opportunistic fungi that cause respiratory infections in dogs which may evolve to systemic disease. Disease in dogs is caused by *A. fumigatus*, *A. terreus*, *A. flavus*, *A. niger*, *A. deflexus*, and *A. caninus*. *Aspergillus fumigatus* and *A. flavus* typically cause nasal cavity infections, while *A. terreus*, *A. deflexus*, and *A. niger* cause systemic disease. Central nervous system involvement may occur due to extension of upper respiratory disease through the cribriform

plate. Widespread disease occurs secondary to transport within leukocytes to other organs or through angioinvasion followed by hematogenous spread. This report describes mycotic meningoencephalitis and pyelonephritis caused by *Aspergillus ochraceus* in a 3-year-old female dog with a one-month history of lethargy, hyporrhexia, bilateral protrusion of the nictitating membrane, and tetraparesis. A brain MRI was consistent with meningoencephalitis of unknown region. Clinical signs continued to worsen despite treatment with cytosar, dexamethasone, clindamycin, and pantoprazole, and the dog was euthanized. The brain and kidneys were grossly unremarkable. Histological findings were granulomatous inflammation in the brain, meninges, and kidneys with intralesional fungal hyphae. Fungal cultures were negative. Pan-fungal PCR confirmed the presence of *Aspergillus ochraceus* in the brain, while no fungal sequence was identified in the kidneys. No previous identification or histological description of *A. ochraceus* in meningoencephalitis was found in the veterinary literature. This distribution suggests that the pathogenesis of this species differs from other species of *Aspergillus*. *A. ochraceus* has non-parallel and beaded walls, which may aid distinguishing this species from other *Aspergillus* spp.

5: THE PATHOLOGY OF ATHEROSCLEROSIS: PLAQUE DEVELOPMENT IN APOLIPOPROTEIN E-DEFICIENT MICE

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Background: Atherosclerosis-prone apolipoprotein E-deficient mice display poor lipoprotein clearance with subsequent accumulation of cholesterol ester-enriched particles in the blood, which promote the development of atherosclerotic plaques.

Objective: National Laboratory Animal Center (NLAC) used CRISPR/Cas9 technology to generate ApoE knockout mouse C57BL/6-Apoe^{em1Narl}/Narl, and validated its phenotypes including high serum cholesterol and atherosclerosis.

Methods: ApoE knockout mice were humanely euthanized and complete post-mortem examination performed. Tissues were fixed in 10% neutral buffered formalin, processed routinely, and stained with histochemical stains (H&E, Masson's trichrome and Von Kossa).

Results: On regular diet, as early as 8 weeks of age, foam cell lesions were observed by light microscopy in our study. Monocytes attachment to endothelial cells are noticed from 8 weeks of age. Intermediate lesions containing foam cells and smooth muscle cells were seen at 15 weeks, and fibrous plaques at 20 weeks of age. During the early stages of lesion formation, lipid-filled macrophages appear in the subendothelium, and accumulate leading to fatty streaks. After 16-20 weeks, intermediate lesions are present extracellular matrix, inflammation and fibrous cap. In more advanced lesions, plaques reveal cholesterol clefts and calcification with time.

Conclusions: Lesion distribution in ApoE^{-/-} mice are similar to humans, with a predominance in the aortic root, carotid artery, and aortic branches. The study provides a histopathological baseline for spontaneous hypercholesterolemia associated with atherosclerosis. ApoE deficient mouse model have provided tremendous insight into atherogenesis, but even they do not perfectly recapitulate the human disease, as they lack plaque rupture and thrombosis.

6: EVALUATION OF THE EFFECTS OF AN ANTI-APOPTOSIS SOLUTION (Q-VD-OPH) ON PRESERVING CANINE NEUTROPHIL FUNCTION AT 4°C

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Background: A universal obstacle of research in neutrophil function, is the limited viability of neutrophils following blood sample collection, leaving a narrow time-window from sample collection to evaluation. A preservative solution (Q-VD-OPh) has been shown to stabilize leukocyte viability in humans over a 72-hour period. However, there remains limited information regarding how well the preservative maintains neutrophil viability and function in canine patients.

Objectives: After determining the optimal concentration of Q-VD-O-Ph to add to whole blood samples, neutrophil respiratory burst and phagocytosis was measured and compared via flow cytometric evaluation in samples of normal canine blood (with and without Q-VD-OPh added) at 0, 24, 48, and 72-hour intervals at 4°C.

Methods: Heparinized blood was collected from 20 healthy dogs. Viability was investigated by multi-color flow cytometry using Zombie Green™ to identify necrotic cells and PerCP/Cy5.5 Annexin V staining to identify apoptotic cells. Neutrophil function was investigated using commercial kits designed for flow cytometric assessment of neutrophil respiratory burst and phagocytic ability.

Results: Neutrophil viability was optimized at 80µm Q-VD-OPh as seen by a significant decrease in cells positive for Zombie Green and Annexin V. There was not a significant difference in neutrophil phagocytic and oxidative burst activities with preservative at 72 hours compared to baseline, but there was a significant decrease without preservative ($P = 0.0002$ and $P = 0.0133$, respectively).

Conclusions: Q-VD-OPh preserves neutrophil viability, phagocytic and oxidative burst activities in canine patients, allowing for increased patient follow-up evaluation and enrolment in studies examining neutrophil function.

7: POSTMORTEM MICROWAVE INJURIES IN RATS

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Background: Acts of animal cruelty include small animals being killed in microwaves. Microwaves injuries occur rarely, and the current literature lacks descriptions of the resulting lesions, challenging forensic investigations and the postmortem examination. Microwaves use electromagnetic radiation to heat solids or liquids by causing their water and other molecules to vibrate and rotate. This cause friction between molecules that produces heat that will cook the content.

Methods: We used 30 freshly euthanized 12-week-old, male rats and microwaved them at maximum power for 30 seconds (n=10), 90 seconds (n=10) and 120 seconds (n=10).

Results: Gross changes were consistent across all animals, with increasing severity at longer microwaving times and included: pugilistic contraction of paws/limbs, exophthalmos with spontaneous ejection of white discolored lenses, unilateral abdominal swelling, curling of lips-nose-ears-tail, subcutaneous air pockets on the tail, skin lacerations, ruptured intestines, cooked appearance of internal organs and skeletal muscles, and melting of adipose tissue. Histologic examination is pending.

Conclusion: Microwave induced lesions are already present after 30 seconds, intensify when microwaved for 90 and 120 seconds, and some resemble those of burn victims. Findings contrasting

burn injuries include the subcutaneous air pockets on the tail, abdominal swelling, skin lacerations, lens extrusion and ruptured intestines. These findings suggest that 30 seconds of microwaving could be deadly and that most of the additional findings are consistent with an “inside to outside” heating process where liquids within the body get hot very fast.

8: CYTOLOGICAL AND HISTOPATHOLOGICAL DESCRIPTION OF OSTEOSARCOMA IN A BEARDED DRAGON (POGONA VITTICEPS)

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A 4 year, 8 months old male Bearded dragon (*Pogona vitticeps*) with a 3-month history of left fore lameness and discomfort was presented to the Exotics service of the Royal Veterinary College. Radiographs revealed an osteolytic lesion on the left distal humerus that failed to respond to treatment with antibiotics and analgesia. An ill-defined, expansile, space occupying soft tissue attenuation was found on CT examination in the distal metaphysis of the left humerus, effacing the expected trabecular pattern of the bone with associated soft tissue swelling. The main differential diagnoses at the time were osteomyelitis vs bone neoplasia and fine needle sampling of the lesion was performed. Cytological examination revealed a population of spindle cells with a moderate degree of nuclear atypia and occasional association with extracellular matrix material (suspected osteoid), alongside well-differentiated osteoblasts and osteoclasts and lack of inflammation. It was interpreted as a mesenchymal proliferation, favoring sarcoma with osteosarcoma being the top differential, based on the combination of cell morphology, clinical signs, imaging findings, and anatomical location. A week later the limb was amputated and submitted for histopathological examination; an osteosarcoma with chondroblastic differentiation was diagnosed. Bone neoplasia in this species is rare with only one other case of osteosarcoma reported previously, which was subclassified as fibroblastic. The current case is unique given that chondroblastic osteosarcoma has not been reported in this species and there is full imaging and cytological characterization with histopathological confirmation of the lesion as well as excellent diagnostic correlation.

9: CYTOLOGIC AND HISTOLOGIC CORRELATES OF ALVEOLAR RHABDOMYOSARCOMA IN A 1-YEAR-OLD GERMAN SHEPHERD DOG

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Introduction: In dogs, rhabdomyosarcomas are rare soft tissue neoplasms that most commonly arise from the skeletal muscle and less commonly from the heart, lung, and urinary bladder. They are often found in the neck and oral cavity of young animals, can be markedly invasive, and can present as one of multiple histological subtypes.

Case history: A 1-year-old, male, German Shepherd dog presented for a progressive submandibular swelling and multiple cutaneous masses. Antemortem cytology and biopsy were suggestive of round cell neoplasia and the dog was euthanized. Necropsy revealed widespread visceral masses.

Results: Cytology of the mandibular mass had high nuclear cellularity with many extruded nuclei. Cells included predominantly large round cells with a small amount of cytoplasm and large round nuclei with stippled to lacy chromatin and variably prominent nucleoli. Histologically, multiple tissues were expanded by well demarcated, densely cellular, unencapsulated masses composed of sheets and short streams of neoplastic cells with scant fibrovascular stroma. Neoplastic cells had a small amount of eosinophilic cytoplasm and large, round to oval nuclei with finely stippled chromatin and

one central nucleolus. There were 64 mitoses in 2.37 mm². Neoplastic cells were immunoreactive to MyoD1, desmin, and muscle-specific actin, and negative for smooth muscle actin, diagnostic for rhabdomyosarcoma.

Conclusion: Most subtypes of rhabdomyosarcoma are not predominated by spindle cells, which often confounds the diagnosis. In this case, the neoplasm had variable embryonal (round cells) and spindle cell/sclerosing morphology; sampling of the former led to suspicion of a round cell tumor on antemortem histopathology.

10: AGREEMENT BETWEEN FLOW CYTOMETRY AND IMMUNOCYTOCHEMISTRY IN DETERMINING KI67% IN CANINE LYMPHOMA

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Background: The nuclear protein Ki67 is a cell proliferation marker usually accessed during the lymphoma diagnostic workflow through immunohistochemistry (IHC) or flow cytometry (FC) according to different methods and cutoff values.

Objective: To determine if Ki67% could be accurately accessed by ICC in canine lymphoma, comparing the degree of agreement between ICC and FC. **Methods:** Thirty-eight cases of canine lymphoma were analyzed by FC and ICC simultaneously and the Ki67% was determined. The correlation between ICC and FC Ki67% values was estimated with Spearman's Rank correlation coefficient. Agreement between methods was assessed by the Bland-Altman difference plot. ROC curve was calculated to identify the best ICC cutoff to discriminate between low- and high-grade forms.

Results: According to the FC cutoff previously described, twenty-three lymphomas were high grade (17 B cell and 6 T cell) and fifteen were low grade (2 B cell, 14 T cell and 1 undefined). Mean Ki67% was 24.9 (range 1.3-85.6) and 28.5 (range 1.0-88.8) on FC and ICC, respectively. Spearman's coefficient of correlation was $p = 0,94$ (95% CI:0,89-0,97). The Bland-Altman plot showed a proportional bias with a mean difference of 3,58 (95% CI:1,24-5,93) and limits of agreement from - 10,38 to 17,55. ROC curve suggest 19.4% as the best ICC value to detect high-grade forms (sensitivity 97.5%; specificity 100%).

Conclusions: These results show a high correlation and agreement between Ki-67 values by FC and ICC, allowing a practical and reliable assessment of lymphoma proliferation activity on cytology samples. Further prognostic studies are needed.

11: ABSENCE OF SPLENOMEGALY AND EXTERNAL HEMORRHAGE IN A WYOMING BOVINE ANTHRAX OUTBREAK

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During the summer of 2024, at least 50 beef cattle mortalities occurred among multiple rangeland herds within an approximately 16-square mile area of southern Wyoming. Mortalities occurred in a metachronous pattern involving single to few cattle over two months, affecting both adults and calves.

The attending veterinarians observed acute-onset but variable and non-specific clinical signs among a small number of cattle observed prior to death, including lethargy, anorexia, and apparent ataxia and/or blindness. The majority of cattle were found dead on extensive rangeland, and were not observed prior to death. On-site necropsies were performed on multiple cattle. Anthrax was not initially suspected due to no known history of anthrax at this location, and an absence of splenomegaly and massive external hemorrhages. Gross necropsy findings included endocardial, splenic capsular, and mesenteric hemorrhages and segmental intestinal mucosal congestion and/or luminal hemorrhages. Histopathology was performed on limited tissues from four cattle. In all four cases, small to large numbers of bacteria morphologically similar in appearance to postmortem bacilli were present in multiple tissues. Evidence of inflammation or necrosis was not present in association with these bacteria with the exception of one of two cases for which spleen was available. In that case, moderately severe splenic necrosis and fibrin deposition accompanied the bacilli. *Bacillus anthracis* was detected by RT-PCR in five tested cattle. This case series demonstrates the importance of considering anthrax in cattle mortality events even in the absence of splenomegaly and external hemorrhage.

13: HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS (H5N1) INDUCED NECROTIZING MENINGOENCEPHALITIS AND BRONCHOINTERSTITIAL PNEUMONIA IN AN INDOOR-HOUSED DOMESTIC SHORTHAIRED CAT IN COLORADO

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Background:

A 4.5-year-old neutered male, indoor-housed Domestic Shorthaired cat (*Felis catus*) presented with a decreased appetite and lethargy. The cat was up to date on vaccinations with no prior health issues reported. Within 48 hours of initial presentation, clinical signs escalated to severe pyrexia, respiratory distress, neurologic deficits, focal and tonic-clonic seizures. Despite intervention, the cat's neurologic condition worsened to a state of obtundation, leading to euthanasia.

Results:

Initial bioanalytic data demonstrated moderate leukopenia, thrombocytopenia, panhypoproteinemia, elevated inducible and leakage hepatic enzymes, and hyperbilirubinemia. Diagnostic imaging showed an interstitial to alveolar pneumonia, and mild, pleocellular pleural and peritoneal effusions. Postmortem gross findings included consolidated lungs with peribronchiolar pallor, multifocal to coalescing hepatic hemorrhage, and multifocal gastrointestinal ulceration. Histopathologic examination revealed necrotizing and neutrophilic meningoencephalitis and necrotizing bronchointerstitial pneumonia alongside necrotic lesions in the vascular, hepatic, lymphoid, pancreatic and gastrointestinal tissues. Influenza A/H5N1 subtype was confirmed in the brain and pulmonary tissues using real-time reverse transcriptase polymerase chain reaction (rt-PCR). Rabies was excluded based on a negative indirect fluorescent antibody test. Feline calicivirus and Feline herpesvirus 1 were not detected in pulmonary tissue via rt-PCR.

Conclusions:

This case highlights a rare instance of influenza A/H5 infection in a domestic feline presenting with severe systemic and neurological manifestations. The findings are consistent with prior case reports of both H5 and H1 HPAI subtype infections in felids and underscore the importance of considering HPAI in differential diagnoses for neurologic presentations in domestic cats regardless of exposure to prototypical hosts.

14: LATERAL VICE CERVICAL DISLOCATION: A NOVEL METHOD FOR SECONDARY PHYSICAL EUTHANASIA IN HAMSTERS

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Hamsters are listed in the header for the laboratory rodent section of the AVMA Euthanasia Guidelines, but without a tail and with thick necks, they are traditionally not considered for cervical dislocation. As there is no guidance on methods for secondary physical euthanasia specific to the species, many institutions provide guidance on guillotine, prolonged CO₂ exposure, or bilateral thoracotomy. A lab investigating RSV in a hamster model requested veterinarian input for a more rapid confirmation of euthanasia due to the delicacy of the tissues for harvest. The lab noted variance in titers and autolysis of tissues following prolonged CO₂ exposure. Hamster carcasses were assessed for novel methods of cervical dislocation, culminating in a lateral vice cervical dislocation (LVCD) method. In deeply anesthetized hamsters, LVCD resulted in immediate cessation of breathing, loss of corneal reflex, voiding, and irregular and rapidly decreasing heartrate. Radiographs showed cervical dislocation and displacement of the resultant vertebral ends without skin penetration. Manual palpation of LVCD hamsters by vet staff resulted in 100% identification of cervically dislocated hamsters when compared to prolonged CO₂ and bilateral thoracotomy controls. On gross necropsy of LVCD hamsters the spinal cord was severed typically at the atlantoaxial joint, and no further distal than C3. We submit this method as a useful secondary physical method with relevance for labs utilizing hamsters in respiratory research as it is trachea sparing and lung sparing, or labs working in BSL2+ as a general method to minimize sharps usage. Manuscript in process.

15: NASAL AND CONJUNCTIVAL CARCINOMAS IN A GREAT HORNED OWL (*BUBO VIRGINIANUS*) ASSOCIATED WITH A NOVEL HERPESVIRUS

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Orthoherpesviridae is a family of large double-stranded DNA viruses known for persistent or latent infections in the clade Amniota (reptiles, birds, and mammals). The great horned owl (GHO)(*Bubo virginianus*) is common throughout the Americas. Herpesvirus infection in GHOW has been documented as the most commonly diagnosed infectious cause of death, with affected birds showing hepatosplenitis with intranuclear inclusions as consistent lesions.

This study characterizes findings associated with a novel alphaherpesvirus in a GHOW with a history of chronic conjunctival and upper respiratory infections that developed local neoplasms. Microscopic examination revealed intranuclear inclusion bodies consistent with herpesviral infections within neoplastic cells.

PCR and phylogenic (Bayesian and Maximum Likelihood) analysis identified the herpesvirus as a novel member of the genus *Iltovirus*, designated as Strigid Alphaherpesvirus 2 (StrAHV2). This is the first report of an *Iltovirus* infecting an owl. The role of StrAHV2 in oncogenesis remains to be determined. Further investigation is required to elucidate whether StrAHV2 infection in GHOW is endemic or aberrant and determine its potential as an oncogenic virus.

17: SUSTAINING THE PATHOLOGY PIPELINE: MODERNA-ACADEMIA TRAINING COLLABORATIONS IN TOXICOLOGIC AND INVESTIGATIVE PATHOLOGY

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The American College of Veterinary Pathologists (ACVP) Strategic Plan includes goals such as "creating outreach opportunities for students to connect with ACVP and the profession," "developing learning strategies for professional development and continuing education for members at various career stages", and "ensuring a healthy conduit for training future pathologists." In the 2023 ACVP Salary and Benefits Survey, 35% of respondents worked at a contract research laboratory or a pharmaceutical company, and 26% of all respondents identified as toxicologic pathologists. To support training institutions in providing engaging and challenging activities for veterinary pathology trainees, Moderna's team of scientists and pathologists has developed comprehensive externship, Co-op, and postdoctoral fellowships in toxicologic pathology and investigative pathology that can benefit undergraduate, graduate, and/or veterinary students, residents, and/or early career pathologists. These programs grant trainees experience via advanced, practical, real-world training that forms a bridge between their academic training and future career in pathology, toxicology, drug development, and/or biomedical research. When these trainees successfully move on to future positions, they will have skills enriched by experts in the field and will have developed confidence and independence evaluating complicated non-clinical data sets and applying molecular, investigative and digital pathology methods. We highlight examples of how veterinary pathologists in industry, as well as their allied colleagues, can improve exposure to toxicologic pathology and drug development, which not only is critical for the ACVP to achieve its Strategic Plan, but also to provide experiences that augment the excellence within ACVP training programs.

18: DIFFUSE LEPTOMENINGEAL OLIGODENDROGLIOMATOSIS IN A GOLDENDOODLE

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A 4-year-old, female spayed, Goldendoodle presented to the Kansas State University Veterinary Health Center with a 2-week history of lethargy, hyporexia, ataxia, and cervical pain. Neurological examination revealed an absent menace response, moderate ataxia, and significant discomfort with cervical ventroflexion and hyperextension. MRI reported multifocal lesions in the cerebrum and spinal cord. The canine was euthanized due to poor prognosis. On autopsy, the subarachnoid space from the brain to the sacral spinal cord was diffusely expanded by abundant mucoid, cloudy, straw-colored fluid. The underlying meninges and spinal cord were multifocally congested with localized hemorrhage. On microscopic evaluation, expanding the leptomeninges and infiltrating into the spinal nerves and ganglia was a moderately cellular, non-encapsulated proliferation of round cells arranged in sheets on a lightly basophilic background. The round cells exhibited moderate anisocytosis and anisokaryosis with 17 mitotic figures per 2.37 mm². Within the cerebellar white matter were foci of gliosis, Purkinje cell necrosis, and white matter vacuolation. Spinal cord white matter had foci demyelination and spheroids. The round cells immunolabel for Olig-2, glial fibrillary acidic protein, and vimentin, but did not immunolabel for pan-cytokeratin, S100, CD3, and CD20, supporting an oligodendrocyte lineage. These findings are consistent with a diagnosis of diffuse leptomeningeal oligodendrogliomatosis.

19: PERFORMANCE EVALUATION OF THE VETSCAN OPTICELL™ – A NOVEL ARTIFICIAL INTELLIGENCE, CARTRIDGE-BASED POINT-OF-CARE HEMATOLOGY ANALYZER – FOR CANINE AND FELINE AUTOMATED COMPLETE BLOOD COUNT.

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Introduction:

The Vetscan OptiCell™ is an automated, 3D-imaging, artificial intelligence-based hematology analyzer employing single-use, self-contained cartridges with microfluidic viscoelastic focusing. It is being introduced to improve hematology workflow for dog and cat automated 22-parameter CBCs.

Objective:

Evaluate analytical performance of the OptiCell, including precision, linearity, and method comparison with the Siemens Advia 2120 for dogs and cats.

Method:

Canine EDTA whole blood samples and tri-level commercial quality control material were used to characterize inter- and intra-assay precision on two OptiCell instruments with randomization for operator, analyzer, day, and control level. Healthy and ill dog (n=113) and cat (n=72) EDTA whole blood was analyzed in parallel with two OptiCell instruments and one Advia 2120 for concordance and bias analysis. Manual 200-cell counts for platelets, reticulocytes, and WBC differential were performed and compared to Advia and OptiCell. Statistical analysis was performed using R 4.3 (The R Foundation) and SAS version 9.4 (Cary, NC, USA).

Results:

Inter- and intra-assay total imprecision was acceptable ($\leq 8.5\%$) for most parameters; with higher (8.6% to 15.4%) imprecision for MON, LYM, BAS at some control levels. For both cats and dogs, most parameters achieved excellent (>0.75) concordance between OptiCell and Advia analyzers, including cat and dog platelets and dog reticulocytes. The OptiCell and Advia showed similar agreement with manual counts for cat platelets (0.79, 0.60), dog monocytes (0.63, 0.70), and dog reticulocytes (0.77, 0.89), respectively.

Conclusion:

The OptiCell performed comparable to the Advia 2120 and produced precise automated hematology results for standard parameters in cats and dogs.

20: ENHANCING EMERGENCY PREPAREDNESS FOR REGULATORY TESTING THROUGH EXERCISE AND INTER-AGENCY COORDINATION

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Background: The Oregon Veterinary Diagnostic Laboratory (OVDL) is an essential resource in the Pacific Northwest and a member of the National Animal Health Laboratory Network. As such, the OVDL must respond to sample surges and test for foreign animal, emerging, and non-traditional diseases, while maintaining routine and regulatory testing activities. The project addressed critical gaps in laboratory emergency preparedness (EP) following models for strategic EP.

Methods: Four cycles of exercises, each with a unique scope, scale and participant roster, were followed by process improvement, training, and work on the EP plan, from development through refinement to implementation and integration into the OVDL's quality management program.

Results: Smaller functional exercises on chronic wasting disease and Eastern equine encephalitis examined processes spanning multiple laboratory sections. A large-scale functional exercise on foot

and mouth disease engaged the entire laboratory and 38 participants and emphasized internal and outward facing communication. A series of state-wide table top exercises on African swine fever, coordinated by state animal health officials, had over 80 participants total and focused on interagency cooperation. After-action review of the exercises and, importantly, real-time occurrences (avian influenza outbreak; cat with plague) was identified as a key step and allowed the OVDL to incorporate feedback from all laboratory members and develop corrective actions that enhanced safety and improved laboratory operations.

Conclusion: The OVDL successfully improved EP for regulatory testing in the context of a statewide emergency response through training, targeted exercises, improved inter-agency coordination, and development of a comprehensive and fully integrated EP plan.

21: CARDIAC INTERVENTRICULAR MYXOSARCOMA IN A DOG

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Cardiac tumors are rare, with hemangiosarcoma and chemodectoma the most prevalent. Surgical removal is often desirable but can be challenging due to anatomical constraints. Myxosarcoma is a fibroblastic cancer that can be locally invasive but seldom metastasizes. When the tumor's location permits, surgical excision is typically curative. A 13-year-old neutered male Cavalier King Charles Spaniel presented to the Cardiology service for a follow-up on myxomatous mitral valve disease and congestive heart failure (CHF). The dog exhibited panting and increased bronchovesicular sounds. Thoracic radiographs revealed cardiomegaly and pulmonary edema. Echocardiography identified a large infiltrative hyperechoic structure within the interventricular septum, extending into the left ventricle. The mass had not been identified earlier, but a review of an echocardiogram from the previous year revealed a heterogeneous appearance in the interventricular septum, where the mass was later found. Due to the mass's location and associated risks, biopsy, surgery, and radiation therapy were not recommended, and the patient was discharged with therapy escalation to control CHF. Two months later, the patient was found deceased at home and brought to the hospital for necropsy. Gross findings included a 2.5 cm x 2.5 cm x 3 cm pale tan nodular mass in the interventricular septum. Histology suggested a diagnosis of myxosarcoma with no evidence of metastasis. The tumor stroma was Alcian blue positive, confirming its mucopolysaccharide nature. Cardiac myxosarcoma is a very rare neoplasm in dogs but should be considered in the differential diagnosis of intracavitary heart masses associated with signs of cardiac obstruction and failure.

22: A COMPUTER AIDED DIAGNOSTICS APPROACH FOR DETECTING XENOBIOTIC-INDUCED HEPATOCELLULAR HYPERTROPHY

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Background

Computer-aided diagnostics (CAD) can support pathologists by enhancing the evaluation of hepatocellular hypertrophy, a common and challenging microscopic finding in toxicology studies. This study aimed to develop and evaluate a CAD method to evaluate hepatocellular hypertrophy in rats.

Methods

Hematoxylin and eosin (H&E) stained rat liver sections were digitized at 40x magnification. The Patholytix AI Workflow (Deciphex Ltd, Dublin, Ireland) was used to annotate and train a convolutional neural network (CNN) for hepatocellular nuclei detection. The model was evaluated using manual annotations and a leave-one-study-out cross-validation across eight studies. Detection of hypertrophy on AI-derived nuclear density maps was compared to diagnoses by three pathologists in control and high-dose groups of four unseen studies.

Results

The model achieved a median sensitivity of 88% (IQR 85-90%) and a median positive predictive value (PPV) of 97% (IQR 96-97%), resulting in an average F1-score of 91% (IQR 90-93%). Interobserver agreement among pathologists in high-dose groups was moderate (median weighted kappa 0.60, range 0.49-0.66). Nuclear density correlated negatively with pathologist hypertrophy grades and liver weight (Pearson's correlation coefficient -0.3), though with substantial overlap between grades. Liver weight showed more frequent high outliers in the normal group, often linked to sinusoidal congestion.

Conclusion

AI-derived nuclear density maps enhance the detection of hepatocellular hypertrophy at a whole slide level, enabling side-by-side comparison of hepatocellular size between control and treated rats. Pathologist assessment remains essential for detecting subtle cases and differentiating hypertrophy from other lesions. Further model refinements are needed to address alterations unrelated to hypertrophy.

23: NOVEL PATHOLOGICAL FINDINGS OF NATURALLY OCCURRING LUMPY SKIN DISEASE EPIDEMIC IN INDIAN CATTLE: TIME TO UPDATE THE EXISTING PATHOLOGY

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Background: Lumpy skin disease virus (LSDV) was first-time reported in Odisha, India in 2019.

Objective: Elucidate LSDV-induced pathology and pathogenesis during various stages of LSD in natural epidemic in Indian cattle.

Methods: Examined 1725 cattle across 75 LSDV outbreaks, 625 were clinically affected, and conducted necropsy of 59 cattle during 2021 to 2024 in different States of India. Grading, staging of skin lesions, gross and histopathology, LSDV confirmation, and immune responses were studied.

Results: LSDV-induced morbidity, mortality and case fatality rates of 36.23%, 3.42% and 9.44%, respectively. Mild, moderate and severe-grade skin lesions were 34.14%, 39.39% and 26.47%, respectively. Early-, mid- and late-stage skin lesions were 20.81%, 42.02% and 37.17%, respectively. Indian-LSDV caused novel pathological lesions in skin and internal organs during different stages. In early-stage, subcutaneous tissue and lymph nodes showed congestion and haemorrhages. Epidermis showed hydrophic degeneration and intraepithelial microvesicle. In mid-stage, skin nodules

were enlarged (>5.0 cm) and distributed throughout body. Subcutaneous tissue showed sero-sanguinous fluid, necrosis, and nodular lesions in digestive and respiratory tracts. Epidermis and lungs showed oval/round intracytoplasmic inclusions, and enlarged round/oval nuclei with marginated chromatin (cellules clavelleuse). In late-stage, skin nodules showed scab and scar formation, sloughing-off necrotic-core forming 'sit-fasts' lesions with maggot wounds. Necrotic nodular, septicaemic lesions and necrotizing vasculitis were noticed in internal organs. LSDV was demonstrated in nodular/scab lesions and internal organs by immunohistochemistry. Th1-type, Th2-type cytokines, and extrinsic pathway of apoptosis played role in LSDV pathogenesis.

Conclusions: These results formed basis for understanding pathogenesis across different stages of LSD.

24: A HIGH-THROUGHPUT, FAST, OIL IMMERSION WHOLE SLIDE IMAGING SYSTEM FOR DIGITAL CLINICAL PATHOLOGY

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Background. High resolution whole slide imaging has the potential to transform how clinical pathologists evaluate glass slides and how they perform cell differentials. Digital pathology is already used for histologic evaluation and in some clinical pathology laboratories. However, the use of whole slide images (WSI) scanned at a suboptimal magnification creates diagnostic challenges. The development of a high-resolution and high-magnification scanners, that are fast and reliable is required.

Objectives. Demonstrate the scanning characteristics of MetaSystems' Metafer Scanner (Meta.MAX).

Material and Methods. Meta.MAX is a scanning system that uses linear and piezoelectric motors to scan slides with a high-magnification, oil immersion objective (100x or 60x). This configuration allows for fast creation of high-resolution images within an automated system. Blood smear (BS), fine needle aspirate (FNA), bone marrow smear (BMS), and cytospin smears (SPIN) were scanned. Image quality was reviewed by a clinical pathologist, time to scan and image size were recorded.

Results. Image quality for all scans was considered equivalent to manual microscopy. Time to scan was fast, with ≤ 6 mins for BMS and BS, ≤ 3 mins for various FNA and ≤ 1.5 mins for SPIN.

Conclusion. Meta.MAX is a high-throughput WSI scanner that uses a unique focus map algorithm and scanning technique to provide WSI using oil immersion objectives, under 6 mins. Deep neural networks and artificial intelligence algorithms can be developed and trained to automate cell differentials. Generated WSI can easily be shared with remote users using MetaSystems' native VSI file format or other neutral file formats.

25: GANGLIONEUROMA IN A MELLER'S CHAMELEON (TRIOCEROS MELLERI)

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Introduction: Nervous system neoplasia is rarely reported in reptiles, with most reports representing gliomas.

Case history: A captive, 9-year-old chameleon was euthanized for weight loss and lethargy. At necropsy, the right caudal coelom contained a 2-cm-diameter mass adhered to the right kidney. The skin had two, 0.8-1.0-cm-diameter, soft, pale-yellow masses over the right leg and left shoulder. The fat bodies were small. A complete tissue set was examined histologically.

Results: The adrenal gland and peri-adrenal soft tissues were effaced by an invasive, moderately cellular neoplasm entrapping remnant chromaffin and interrenal cells and invading the kidney. The neoplasm was composed of two cell populations: well differentiated neurons and peripheral nerves. Cellular atypia was minimal. The skin was multifocally expanded by invasive, unencapsulated neoplasms composed of intersecting streams of neoplastic spindle cells with abundant eosinophilic, finely vacuolated cytoplasm, oval nuclei, marked anisocytosis and anisokaryosis, and occasional mitoses. Neoplastic cells were admixed with few iridophores and melanophores. The fat bodies had marked atrophy. Immunohistochemistry was non-contributory (GFAP, NSE, synaptophysin, NeuN, chromogranin, S100, and vimentin) or negative (AE1/AE3) in neoplastic cells.

Discussion: The adrenal gland mass is a ganglioneuroma, which is a neoplasm of the peripheral nervous system originating from the sympathetic ganglia of the autonomic nervous system. Ganglioneuroma is rarely reported in veterinary species, and this is the first report in a reptile. The origin of this ganglioneuroma was likely neural crest cells of the adrenal gland. It is unknown whether the cutaneous masses were related to the ganglioneuroma or were unrelated sarcomas.

26: HEPATIC HEMANGIOSARCOMA WITH CAVAL INVASION RESULTING IN BUDD-CHIARI-LIKE SYNDROME IN A DOG

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Budd-Chiari-like syndrome is used to describe the clinical signs associated with mechanical obstruction of the post-sinusoidal hepatic veins, caudal vena cava, and the right atrium. To our knowledge, this is the first reported case of Budd-Chiari-like syndrome due to hepatic hemangiosarcoma.

A six-year, seven-month-old neutered male French bulldog was presented for post-mortem examination with a recent history of abdominal distension with serohemorrhagic fluid. Computed tomography identified a large, irregular hepatic mass, infiltrating and markedly expanding the post-hepatic caudal vena cava, obstructing the lumen, and extending to the right atrium. Multifocal pulmonic nodules were observed.

Post-mortem examination identified a 7 x 8 x 5 cm, dark red, soft, blood-filled, spherical to irregular mass arising from the liver. The post-hepatic caudal vena cava measured up to 3 cm in width, and within the lumen was abundant friable, pink-red-dark red tissue contiguous with the hepatic mass. There were a low number of soft nodules on the lung lobe borders, and a 5 mm diameter, soft red nodule on the mucocutaneous junction of the right mandibular lip. Histopathology of the hepatic/intra-caval mass identified an infiltrative mesenchymal neoplasm forming irregular vascular channels. Cells were pleomorphic with marked anisocytosis and anisokaryosis. There was multifocal hemorrhage, necrosis, and intravascular fibrin thrombosis within the neoplasm. The lip and lung nodules showed similar cellular features. Neoplastic cells were immunopositive for vimentin and CD31, and immunonegative for pan-cytokeratin and Iba1, supporting a diagnosis of hemangiosarcoma.

Determination of the primary hemangiosarcoma (hepatic or mucocutaneous) is unclear. Multicentric hemangiosarcoma development is considered.

27: TUBERCULOSIS DUE TO MYCOBACTERIUM BOVIS IN A SHEEP IN A LOW-RISK AREA OF ENGLAND

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Tuberculosis (TB) due to infection with *Mycobacterium (M.) bovis* in cattle is common and of economic significance in England. Conversely, cases in sheep are uncommon and thought to represent spillover of infection. We present a case of *M. bovis*-TB in a sheep residing in a low-risk area of England.

An aged ewe on a unit with dairy cattle, sheep, goats and alpacas presented with dyspnea, harsh lung sounds, pyrexia and poor body condition. Given the poor prognosis, the ewe was euthanized and submitted for post-mortem examination.

The caudal lung lobes were firm with numerous multifocal nodules with cores of caseous necrosis. Additionally, there was chronic pleuritis, and marked mediastinal and tracheobronchial lymphadenomegaly, with nodal effacement by caseous necrotic debris within a fibrous capsule.

Histopathology identified chronic pulmonary and nodal granulomas with fibrous encapsulation, epithelioid macrophages, Langhans multinucleated giant cells, caseous necrosis and mineralization, but acid-fast bacteria were not seen on Ziehl-Neelsen staining. Regardless, the case was notified as suspect TB. A PCR-positive result for *M. bovis* was obtained on lung/nodal tissue and confirmed on culture.

Three skin test reactors, two with TB-compatible lesions, were found in the flock; no reactors were identified in other species on the farm. This ewe entered the flock seven years previously and genomic sequencing (clade B6-62) suggested it became infected at a previous holding. This report highlights an unexpected case of ovine TB, a notifiable and zoonotic disease, but also the low risk of onward transmission of *M. bovis* from sheep.

28: DIAGNOSTIC ASSESSMENT OF A NEW VERSION OF THE VETSCAN IMAGYST® BLOOD SMEAR APPLICATION, A POINT-OF-CARE SCANNING SYSTEM INTEGRATED WITH DEEP-LEARNING ALGORITHMS FOR CANINE/FELINE BLOOD FILM EVALUATION

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Background

Comprehensive hematologic assessments in canines and felines involve both quantitative (cell counts) and qualitative (blood smear) analyses. Analyzing blood smears pose challenges due to technique variations, training disparities, workflow complexities, and time constraints impeding routine blood smears review.

Objectives

Assess the performance of Vetscan Imagyst® (VS-I) Blood Smear Version 2.0, an artificial intelligence algorithm designed for hematologic analysis and compare it to ACVP board-certified clinical pathologists (CPs). Objectives included: determination of WBC estimate, WBC differential, polychromatophil count, nucleated RBC count, and platelet estimate.

Method

Blood smears (119 total, 59 dogs, 60 cats) were retrospectively collected from Zoetis Reference Labs. The Grundium Ocus 40 was used to scan all slides. A randomized 2 out of 4 CPs and the VS-I blood smear v2.0 algorithm evaluated the samples. The agreement between VS-I and the CPs was assessed.

Results

The percentage of samples where VS-I was within 99% prediction interval with the CPs for each white blood cell class differential ranged from 89.9%-100% for dogs and 85.0%-100% for cats.

The number of samples where VS-I was within 95% prediction interval with the CPs for each cell class estimated number ranged from 88.1 – 96.6% for dogs and 88.3 – 96.6% for cats.

Conclusion/Significance

The VS-I Blood Smear v2.0 application evaluation of the blood smears provided cell count and differential results comparable to ACVP-board-certified clinical pathologists hematologic assessments. These findings are supportive of making this application a tool for utilization by veterinarians to obtain a comprehensive CBC.

29: ACCURACY ASSESSMENT OF THE VETSCAN IMAGYST® BLOOD SMEAR APPLICATION FOR IDENTIFICATION AND QUANTIFICATION OF BAND NEUTROPHILS IN CANINE AND FELINE WBC DIFFERENTIALS

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Background

Accurate identification and quantification of band neutrophils in peripheral blood smears (PBS) is vital for characterizing systemic inflammation. However, technique variations, training disparities, workflow complexities, and time constraints often hamper routine in-clinic PBS white blood cell (WBC) morphology evaluations.

Objectives

Evaluate the performance of Vetscan Imagyst (VS-I) Blood Smear Version 2.0, an artificial intelligence algorithm designed for hematologic analysis, in the evaluation of a 6-way differential count (band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils) compared to ACVP board-certified clinical pathologists (CPs).

Methods

Ninety blood smear slides (45 dogs, 45 cats) were retrospectively collected from Zoetis Virtual Laboratory submissions. The Grundium-Ocus 40 microscope-scanner scanned all slides. A randomized selection of 2 out of 3 CPs and the VS-I algorithm evaluated the samples. The agreement between VS-I and CPs and the correlation between CPs, and CPs to VS-I were assessed.

Results

The percentage of samples where VSI was within the 99% prediction interval with CPs for each WBC class differential count ranged from 84.4%-100% for dogs (band neutrophils - 88.9%); 88.9%-100% for cats (band neutrophils – 95.6%); and 86.7% to 100% across species (band neutrophils – 92.2%). Correlations of results between CPs for combined species ranged from 0.792 to 0.998; correlations of results between CP and VS-I for combined species ranged from 0.719 to 0.981.

Conclusion

The VS-I Blood Smear demonstrates alignment with CPs in identifying and quantifying band neutrophils and the other differential elements in canine and feline samples. These findings demonstrate how VS-I aids in comprehensive CBC assessments.

30: ACCURACY ASSESSMENT OF THE VETSCAN IMAGYST® BLOOD SMEARS APPLICATION FOR IDENTIFICATION OF POIKILOCYTOSIS IN CANINE AND FELINE PERIPHERAL BLOOD SMEAR EVALUATION

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Background

The qualitative evaluation of the morphology of red blood cells on a peripheral blood smear (PBS) can offer significant insights into a range of potential conditions, as these morphologic anomalies can stem from pathologic, physiological, or artifactual processes.

Objectives

Assess the performance of the Vetscan Imagyst® (VS-I) Blood Smear Version 2.0, an artificial intelligence algorithm designed for hematologic analysis, in identifying poikilocytes (acanthocytes, echinocytes, eccentrocytes, keratocytes, and schistocytes) on a PBS compared to ACVP board-certified clinical pathologists (CPs).

Methods

Two hundred and thirty-one blood smear slides (129 dogs and 102 cats) were retrospectively collected from Zoetis Virtual Labs submissions. The Grundium Ocus 40 microscope-scanner scanned all slides. Three CPs and the VS-I algorithm evaluated four pre-determined field of views placed on every test slide. The agreement between VSI and the CPs was assessed.

Results

The VS-I AI Blood Smear v2.0 algorithm reliably identified the outlined poikilocytes. For dogs, on a slide level, the sensitivity ranged from 79.3%-100%, the specificity ranged from 80.3%-94.4%, and the accuracy ranged from 79.8%-96.1%. For cats, on a slide level, the sensitivity ranged from 83.3%-93.5%, the specificity ranged from 80.3%-98.7%, and the accuracy ranged from 84.3%-95.1%. For combined species, on a slide level, the sensitivity ranged from 85.0%-97.1%, the specificity ranged from 82.8%-96.4%, and the accuracy ranged from 84.4%-94.8%.

Conclusion/Significance

The VS-I Blood Smear application shows comparable performance in detecting poikilocytosis in canine and feline samples on a slide level when compared to CPs. These findings demonstrate how VS-I aids in agile comprehensive CBC assessments.

31: OPHIDIOMYCOSIS AND OTHER CAUSES AND CONTRIBUTORS TO MORTALITY IN FREE-RANGING EASTERN DIAMONDBACK RATTLESNAKES (CROTALUS ADAMANTEUS) IN THE SOUTHEASTERN UNITED STATES

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Background: The eastern diamondback rattlesnake (EDB, *Crotalus adamanteus*) is a focal species of conservation concern in the southeastern United States. Population declines have been exacerbated by severe human persecution and of more recent concern, threats posed by emerging diseases (e.g., ophidiomycosis) and climate and landscape changes.

Objective: To characterize morbidity and mortality causes and contributors among nine EDB carcasses collected from September 2023 to May 2024 and evaluated at the Southeastern Cooperative Wildlife Disease Study.

Methods: Seven EDBs from Parris Island, South Carolina and two EDBs from Onslow County, North Carolina underwent full postmortem evaluation and gross, histologic, and ancillary results were summarized.

Results: Five females, three males, and one of unknown sex were examined. Seven were adults, one was a subadult, and one was a neonate. The most common diagnosis was ophidiomycosis of which six of the nine EDBs had *Ophidiomyces ophidiicola* (Oo; causative agent of ophidiomycosis) detected via qPCR; five of these had fungal hyphae on histology. Two had systemic fungal infections with hyphae and Oo detected in internal organs (lungs, ova, and kidney). Additional postmortem findings included multisystemic bacterial infections (n=1), goiter (n=3), severe gastrointestinal *Kalicephalus* sp. parasitism (n=2), poor nutritional condition (n=5), blunt force trauma (n=3), and thermal burn injury (n=1).

Conclusions: Morbidity and mortality in wild EDBs are often associated with ophidiomycosis although comorbidities occur and cause of death often is multifactorial. Opportunistic, complete diagnostic examination in species of conservation concern provides vital information to inform conservation management actions such as reintroduction and release programs.

32: CYSTIC RETE TESTIS IN A BEARDED DRAGON (POGONA VITTICEPS)

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Introduction: Testicular lesions are occasionally described in reptiles. Cystic testicular lesions have been described grossly, but not histologically, to the authors' knowledge. There are no publications on cystic rete testis in any non-mammalian species, and <10 case reports in veterinary species, including a cat, fox, horse, alpaca, bull, rabbit, and rats.

Methods: An 11-year-old, male bearded dragon (*Pogona vitticeps*) was evaluated for a coelomic mass. The animal had no other clinical signs aside from coelomic mass effect. Surgical excision was performed, and the mass was submitted for histology. The bearded dragon has no post-operative clinical abnormalities.

Results: Grossly, the 6-cm-diameter, smooth, yellow mass was composed of numerous, 0.5-3.0-cm-diameter cysts filled with yellow fluid. Histologically, cysts were lined by simple cuboidal to flattened epithelial cells that rarely formed small tufts or papillary projections. Cyst lumina occasionally connected with seminiferous tubules, approximately 10% of which were dilated and all of which had normal spermatogenesis. Epithelial cells had a small amount of eosinophilic, slightly vacuolated cytoplasm, rare apical cilia, and basilar, round nuclei with coarse chromatin and small, distinct nucleoli.

Conclusion: This is the first description of a cystic rete testis in a reptile or any non-mammalian species. Cystic rete testis can be primary or secondary to obstruction of the efferent ductules or epididymis. The lack of inflammation and lack of diffuse dilation of the seminiferous tubules suggests that spermatozoa were able to escape, consistent with primary cystic rete testis.

33: DEEP LEARNING ARTIFICIAL INTELLIGENCE (AI) BASED APPROACH FOR EFFICIENT EVALUATION OF CANINE/FELINE DERMATOLOGIC CYTOLOGY SAMPLES

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Background:

Dermatology is a top reason for veterinary visits for dogs/cats, and diagnostic testing is a vital part of case management. A digital tool for rapid, consistent evaluation of dermatologic cytology samples has historically been lacking.

Objective:

The Vetscan Imagyst® (VS-I) AI Dermatology application utilizing an evolved algorithm (v2.0) will accurately identify inflammatory leukocytes, bacteria, and yeast on digital slides in agreement with ACVP-boarded (ACVP) clinical pathologists (CPs).

Methods:

Slides (ear swabs, skin swabs, skin impressions; n=218) from general practice veterinarians and boarded veterinary dermatologists were prepared in-clinic, quick Romanowsky stained, coverslipped, and scanned by Grundium Ocus 40. Slides represented a mix of client-owned canine and feline samples. No animals were utilized in this study. Three ACVP CPs independently scored slides as positive or negative for the presence or absence of object classes: inflammatory leukocytes (neutrophils, macrophages, lymphocytes, eosinophils), bacteria (cocci, bacilli), and yeast (*Malassezia* spp.). VS-I AI Dermatology application analyzed these same 218 slides for presence/absence of each object class.

Results:

The VS-I AI Dermatology v2.0 algorithm reliably identified bacteria (cocci and rods), yeast, and inflammatory leukocytes. Sensitivity for object classes ranged from 77% - 96%; specificity ranged from 76% - 95%.

Conclusions:

Performance of the VS-I AI Dermatology v2.0 algorithm was comparable to expert ACVP

CPs in identification of object classes in dermatology cytology samples. In-house utilization of the Vetscan Imagyst AI Dermatology v2.0 application can provide a diagnostic tool for consistently identifying inflammatory cells, bacteria, and yeast.

34: SERUM MICRORNA BIOMARKERS FOR THE DIAGNOSIS OF SEPSIS IN HORSES

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Background: Sepsis is a severe and often fatal condition in horses characterized by an exaggerated systemic inflammatory response to infection, frequently arising from gastrointestinal diseases that compromise mucosal integrity and facilitate bacterial and lipopolysaccharide (LPS) translocation. Gram-negative bacteria-derived LPS in the blood (endotoxemia) is linked to increased mortality. Despite advancements in diagnostics in human and veterinary medicine, sepsis diagnosis remains

challenging. This study explores the potential of microRNAs as diagnostic biomarkers for sepsis in horses, aiming to provide a rapid alternative to conventional microbiological testing. Material and **Methods:** Total RNA was extracted from serum samples obtained from six healthy horses before and one-hour post-intravenous LPS infusion (paired samples) using the miRNeasy Serum/Plasma Advanced Kit (QIAGEN). RNA was converted into miRNA sequencing libraries using the QIAseq miRNA Library Kit (QIAGEN) and sequenced on a NextSeq 2000 (Illumina Inc.). Pathway analysis was performed using the QIAGEN Ingenuity Pathway Analysis to determine causal effects between genes and functions. **Results:** Differential expression analysis of paired pre- and post-LPS infusion samples identified a total of 686 microRNAs of which 78 showed differential expression at a false discovery rate (FDR) < 0.05 and 21 showed significant differential expression at FDR < 0.01. Pathways related to the regulation of innate and adaptive inflammation and cytokine production were identified. **Conclusions:** These findings reveal specific miRNAs associated with the response to LPS infusion. This study is pioneering in demonstrating altered miRNA profiles in equine sepsis and provides a foundation for future research in both basic science and clinical applications.

35: CYTOLOGIC AND HISTOLOGIC FEATURES OF AN UNCOMMON DISSEMINATED FUNGAL INFECTION TALAROMYCES AURANTIACUS IN A LABRADOR RETRIEVER CROSS WITH CONCURRENT LYMPHOPROLIFERATIVE DISEASE

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A 6-year-old spayed female Labrador Retriever cross was presented for progressive neurological signs, dull mentation, blindness, and generalized lymphadenopathy. A serum biochemistry profile showed hyperproteinemia (10.7, RI: 5.10 - 7.10 g/dL), mild hypoalbuminemia (2.9, RI: 3.3 - 4.2 g/dL), and marked hyperglobulinemia (7.8, RI: 1.70 - 3.40 g/dL). Cytology of the popliteal lymph node revealed occasional extracellular and intracellular hyphae and yeast-like structures. Hyphae were non-pigmented, approximately 2-4µ in width with regular septations, with rare 45-90° branching, most consistent with hyalohyphomycosis. Mild pyogranulomatous inflammation and mildly increased numbers of large lymphocytes and plasma cells consistent with hyperplasia were noted. Despite therapy with voriconazole and antiseizure medications, the dog's condition declined and the owner elected euthanasia. Fungal culture produced three colonies that could not be identified by morphological characteristics or matrix-assisted laser desorption ionization-time of flight mass spectrometry. Sequencing of the 18S rRNA amplicon using fungal culture material revealed forward and reverse sequences identical to *Talaromyces aurantiacus*. Histopathology confirmed the presence of fungal organisms in brain, lungs, kidney, liver, and lymph nodes. These structures were positive for Grocott's methenamine silver and Periodic acid-Schiff stains. In addition, a proliferative lymphoid population was noted in the spleen and lymph nodes. Polymerase chain reaction for antigen receptor rearrangements revealed clonal rearrangement for the immunoglobulin gene, suggesting that the neoplastic cells were B-cell or plasma cell in origin. We hypothesize that the underlying neoplasm led to severe immunosuppression and disseminated fungal infection, although neoplastic transformation of lymphocytes due to chronic inflammation cannot be excluded.

36: NEUROFILAMENT LIGHT CHAIN (NFL) LEVELS IN SERUM ARE A SUITABLE INTRA VITAM MAKER FOR AXONAL DAMAGE IN TMEV-INFECTED MICE

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Neurofilament light chain (Nfl) is a cytoskeletal protein that is highly specific for neurons and is mainly found in large caliber myelinated axons. Damage to axons caused by inflammation, traumatic injury, cerebrovascular diseases or neurodegeneration leads to the release of Nfl into both the cerebrospinal fluid (CSF) and the blood stream. Theiler's murine encephalomyelitis virus (TMEV) causes TMEV-induced demyelinating disease (TMEV-IDD) in SJL mice. Due to the immunological and morphological similarities of the CNS lesions, it is used as a well-established animal model for human multiple sclerosis (MS). TMEV-resistant mouse strains such as C57BL/6 mice usually clear the virus from the CNS within 14 days post infection (dpi). It is hypothesized that serum-Nfl represents a suitable *in vivo* biomarker for monitoring axonopathies in TMEV-IDD.

The aim of this study was to correlate histopathologically evaluated axonal damage in SJL and C57BL/6 mice to serum levels of Nfl at 7, 14, 42 and 147 days post TMEV-infection (dpi). Serum-Nfl was measured using Multi-Array® plates, Meso Scale Discovery. Axonal damage was evaluated by immunohistochemical detection of β -amyloid precursor protein (β -APP) and synaptophysin (syn). Our results show a correlation between axonal damage and serum levels of Nfl in SJL mice. Thus, Nfl is a promising marker for the *in vivo* evaluation of axonal damage in TMEV-infected mice.

37: EXPLORING THE ROLE OF PARACRINE SIGNALING DURING MIGRATION OF THE DROSOPHILA SALIVARY GLAND

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Background: The fruit fly (*Drosophila melanogaster*) has long been utilized for understanding complex signaling transduction pathways. A popular invertebrate model with numerous tools for genetic manipulation, it is ideal for uncovering the molecular basis of congenital disease. Our laboratory employs the embryonic salivary gland (SG), visible through the use of immunohistochemical staining, as a model of simple epithelial tube formation analogous to the mammalian lungs and glandular tissues.

Objective: Though our laboratory has determined the tissue substrates that mediate posterior SG migration, questions regarding the paracrine signaling interactions that contribute to this process remain. We hypothesize that using our published scRNA-seq data, we will discover ligand and receptor pair(s) that work together to control SG migration along the circular visceral mesoderm (cVM).

Methods: Using manual and computational analysis of our wild-type whole embryo scRNA-seq data in comparison to a web-based repository of intra- and intercellular signaling molecules in *Drosophila*, we identified candidate molecules. We now seek to determine if existing loss-of-function mutations in these molecules disrupt SG placement.

Results: Our work reveals 48 unique signaling receptors expressed in the SG, with many dedicated to GPCR or Wnt-related pathways. Through phenotypic assessments of an initial pool of null mutants, we will ask if loss of these genes alter or prevent SG migration.

Conclusions: Here, we show how scRNA-seq datasets can be utilized to predict ligand-receptor interactions mediating morphogenetic processes from gene expression in their corresponding cell-specific clusters. This allows us to explore the function of these candidate molecules through genetic modification.

38: DIAGNOSTIC ACCURACY OF A RAPID IMMUNOASSAY FOR THE DETECTION OF BACTERIA IN CLINICALLY SUSPECTED SEPTIC PERITONEAL AND THORACIC EFFUSIONS IN DOGS

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Background: Bacterial culture is considered the gold standard for identification of a septic effusion, but it takes several days to perform. While cytologic identification of bacteria in effusions provides fast results and is highly specific, overall sensitivity is variable. A lateral flow rapid immunoassay (RIA) originally developed for point-of-care detection of canine urinary tract infections is reported to have high sensitivity and specificity when compared to urine culture. When previously comparing RIA use with all types of canine peritoneal effusions to cytology and culture, the RIA was found to have moderate accuracy with similar sensitivity to cytology and bacterial culture, but lower specificity. Our study seeks to investigate the accuracy of the RIA (compared to bacterial culture) only in cases in which a septic effusion is a top differential.

Objective: To investigate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the RIA compared to bacterial culture for detection of bacteria in dogs clinically suspected to have septic peritoneal and/or thoracic effusions.

Methods: Body cavity effusions will be aseptically evaluated via RIA using manufacturer-provided instructions, and results will be compared to aerobic bacterial cultures.

Results: To date, 24 test comparisons have been performed with sixteen (66.7%) true positives, seven (29.2%) false positives, one (4.1%) true negative, and zero false negatives. As a result: sensitivity = 100%, specificity = 12.5%, PPV = 69.6%, NPV = 100%, accuracy = 70.8%.

Conclusions: The RIA provides excellent sensitivity and NPV, but overall low PPV, accuracy, and very low specificity.

39: QUANTIFICATION AND PROGNOSTIC SIGNIFICANCE OF CD204+ TUMOR-ASSOCIATED MACROPHAGES IN CANINE APPENDICULAR OSTEOSARCOMA PATIENTS RECEIVING STANDARD-OF-CARE TREATMENT

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Background: Osteosarcoma is the most common bone tumor of dogs, with an aggressive clinical course and poor prognosis. Components of the tumor immune microenvironment are of interest for their potential use as prognostic biomarkers, role in disease progression, and utility as therapeutic targets. Tumor-associated macrophages (TAMs) are one component with a spectrum of tumor-promoting and -inhibiting effects. A previous study in canine osteosarcoma suggests an inhibitory role of CD204+ TAMs in the progression of metastasis. Another has shown that CD204+ TAM levels are comparable between primary and metastatic tumors.

Objective: We aimed to quantify CD204+ TAMs in canine appendicular osteosarcoma through digital image analysis and determine if they correlate with outcome in patients receiving standard-of-care treatment.

Results: CD204+ TAMs were labelled in the histologic sections of 28 cases using immunohistochemistry and analyzed in QuPATH. A pixel-based classifier was developed to generate 3 annotation classes: CD204+ TAMs, hematoxylin-stained tumour tissue, and debris/artifacts. The CD204+ area ranged from 0.0-4.2%, the median value was 0.4%, and 7/28 cases had a positive area of 1% or greater. Kaplan-Meier curves for disease-free interval and survival time were compared by log-rank test. CD204+ area did not significantly correlate with either.

Conclusions: CD204+ TAM area was less than 1% in most cases. No correlations with patient outcome were identified on survival analysis. This contrasts with the previous literature. Correlations of CD204+ TAMs with other biological features may better define their role in disease progression beyond prognostic significance.

40: DIFFUSE LARGE B-CELL LYMPHOMA WITH MOTT CELLS IN THE ILEOCECOCOLIC JUNCTION OF A CAT

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Background: The most common feline enteric round cell neoplasms are enteropathy-associated T-cell lymphoma and diffuse large B-cell lymphoma (DLBCL). DLBCL is composed of sheets of monomorphic, intermediate to large lymphocytes.

Case history: A 14-year-old Maine coon cat had weakness, weight loss, and a palpable mass in the caudal abdomen. The animal was euthanized, and necropsy revealed a 7x7x4-cm, transmural mass at the ileocecolic junction that was submitted for pathology.

Results: Infiltrating the ileocecolic junction and adjacent lymph node is a neoplasm composed of sheets of heterogeneous lymphocytes, with areas of both low-grade (small cells, 4-6 mitoses per 0.237 mm²) and high grade (intermediate cells, 6-10 mitoses per 0.237 mm²). Throughout the neoplasm, up to 20% of cells are Mott cells with abundant, PAS-positive, cytoplasmic globules. Neoplastic lymphocytes and Mott cells are immunoreactive to CD20 and PAX5, and negative for reactivity to MUM-1 and CD3. Mott cells also have strong stippled cytoplasmic immunoreactivity to IgG, and weak stippled to diffuse cytoplasmic immunoreactivity to kappa and lambda light chains, IgM, and IgA.

Conclusions: Mott cells are not a reported feature of DLBCL. The Mott cells in this case may either represent neoplastic lymphocytes (i.e., lymphoma with Mott cell differentiation) or reactive Mott cells due to long standing enteritis. Lymphoma with Mott cell differentiation is rarely reported in cats and dogs, but is not currently recognized in the WHO classification of lymphoid neoplasms in dogs and cats. Myeloma-related disorders were excluded based on the lack of MUM-1 immunoreactivity.

41: FATAL SYSTEMIC TRYPANOSOMIASIS WITH SEMINIFEROUS TUBULE INFILTRATION IN A NATURALLY INFECTED DOG

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Background: Canine trypanosomiasis, caused by protozoal *Trypanosoma cruzi*, is an endemic disease in southern United States. Acute and chronic illness has been described, most commonly in young dogs, and death from severe myocarditis. *T. cruzi* amastigotes commonly infect and incite profound inflammation in multiple tissues. These parasites have been reported in the seminiferous tubules of experimentally infected mice. To our knowledge, this is the first report of *T. cruzi* amastigotes within the seminiferous tubules of a naturally infected canine.

Methods: A 1.5-year-old male Belgian Malinois, previously healthy, was found unresponsive following exercise and a one-day history of mild diarrhea. CPR was unsuccessful. A full necropsy revealed mild enlargement of the right atrium; multiple random, pinpoint, white foci on the capsular surface and extending into cut section of the kidneys; and similar foci in the lungs. Following routine histologic evaluation, transmission electron microscopy, histochemical stains (GMS and PAS), immunohistochemistry (*T. cruzi* antibody), and PCR (*Leishmania* spp.) were performed.

Results: Histologically, there is severe lymphohistiocytic inflammation in multiple tissues to include the heart and testes. Occasional clusters of 3µm amastigotes are rarely scattered extracellularly and within macrophages and possibly seminiferous epithelial cells in seminiferous tubules. Amastigotes are positive for *T. cruzi* on immunohistochemistry. Amastigotes are also rarely identified within cardiomyocytes. PCR and histochemical stains are negative.

Conclusions: This is the first report of *T. cruzi* within the seminiferous tubules of a naturally infected canine. This may have implications for vertical transmission within the canine population in endemic areas.

42: PRECURSOR-TARGETED IMMUNE-MEDIATED ANEMIA IN A DOMESTIC CAT

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Background: PIMA is characterized by nonregenerative persistent anemia with variable BM findings, including rubriphagocytosis, and the absence of peripheral RBC destruction. It has been rarely described in cats, and although its pathogenesis is still unclear, an immune mediated mechanism is presumed. There is a lack of standardization in the diagnosis and assessment of BM morphology in cats.

Objective: Report of CBC and BM findings in a case of PIMA in a cat.

Methods: EDTA blood and BM samples were obtained from an eight-year-old domestic short hair cat presented with two episodes of non-regenerative anemia responsive to immunosuppressive therapy over the past 3 years. Wright-Giemsa-stained BM smears were evaluated. PCR for FIV, FeLV and Mycoplasma spp. were negative.

Results: At presentation, a non-regenerative, normocytic normochromic anemia (16% PCV, 4.8 g/dL HGB, 28,800/µL aggregate RETs), and hyperglobulinemia (5.99 g/dL) were observed. BM cytology was hypercellular (95% cellularity), M:E ratio of 0.19, erythroid hyperplasia with a mild left shift (7.4% prorubricytes, 10% basophilic rubricytes, and 9.8% polychromatophilic rubricytes), and marked dysplasia in >10% of these cells. There was evidence of an increased number of macrophages with phagocytosis of intact rubricytes and plasmacytic hyperplasia (4.5%). Myeloid and megakaryocytic lineages were unremarkable. The cat received prednisolone 2.5 mg/kg/SID and the PCV increased to 35% within 30 days.

Conclusion: The diagnosis of PIMA in cats is challenging due to the limited reports on its clinical and laboratory presentation. The differential diagnosis between PIMA and PRCA should be considered in cats with non-regenerative anemia.

43: HEMATOLOGIC VALUES IN GREATER SAGE-GROUSE (CENTROCERCUS UROPHASIANUS) BLOOD SAMPLES ANTICOAGULATED WITH LITHIUM HEPARIN VERSUS

ETHYLENEDIAMINETETRAACETIC ACID (EDTA), WITH COMPARISON OF DIFFERENT ANTICOAGULANT-STAIN COMBINATIONS FOR BLOOD SMEAR EVALUATIONS.

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Background: The Wilder Institute-Calgary Zoo has Canada's only conservation breeding for release program for endangered Greater sage-grouse (*Centrocercus urophasianus*). Health monitoring involves hematological evaluation, yet best anticoagulant and hematology stain combination is unknown.

Objectives: Compare PCV and TS between different anticoagulated samples; assess leukocyte morphology in Wright-Giemsa (WG) and quick dip (DQ) stained smears from each.

Methods: PCV and TS were manually measured in EDTA and lithium heparin (LH) samples from 35 adult birds. Blood smears from each anticoagulated sample stained with WG and DQ were assessed for leukocyte staining quality, clumping, cell lysis and differential counts by novice and expert raters.

Results: Mean TS EDTA was 56.29 +/- 7.478 versus LH 53.14 +/- 6.607 g/L (P -value 0.0001); mean PCV was not significantly different. Leukocyte staining was adequate in most EDTA-WG and LH-WG smears per both novice (23/26 (88.46%), 25/26 (96.15%) and expert raters (25/26 (96.15%), 23/26 (88.46%) compared to EDTA-DQ and LH-DQ (novice: 6/26 (23.08%), 3/26 (11.54%), experts: 10/26 (38.46%), 13/26 (50.0%), P -value <0.0001). Significant variation in mean lymphocyte percentage was found by both novice (F-statistic 5.038, P -value 0.0095) and experts (F-statistic 5.850, P -value 0.0022); post-hoc analysis showed differences between novice LH-DQ versus EDTA-DQ (32.09 +/- 19.38, 24.74 +/- 14.43, P -value 0.0113) and experts LH-DQ versus LH-WG (35.83 +/- 17.41, 27.43 +/- 15.95, P -value 0.0024). No other comparisons detected significant differences.

Conclusions: Significant variation in lymphocyte percentage may exist in LH-DQ prepared sage-grouse smears; WG may provide better quality leukocyte staining. TS measurement may be increased in EDTA versus LH samples.

44: TIME AND MOTION STUDY: FORESIGHT V1 ANALYSIS OF EFFECTIVENESS AS AN AI DECISION SUPPORT TOOL

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Introduction

This study evaluated the impact of the Foresight AI decision support tool on the efficiency of pathologists when interpreting preclinical toxicology studies. This study aimed to identify both the quantitative and qualitative differences when reading toxicology studies with and without Foresight.

Methods

The study was conducted over four months, with six pathologists. A pseudo-study of 105 slides was created, which contained 15 slides for each of seven organs which the Foresight tool covers. Each organ had three groups, control, mid-dose and high-dose. These slides were collected from multiple rodent studies.

The six pathologists were asked to read this study and provide findings and gradings for each slide. They tracked their findings using a standard data capture system and time taken for review using a free time logging software. The washout period of two months was introduced, at the end of which the

pathologists were trained on how to use the decision support tool. All the pathologists repeated the same study review with the availability of the AI decision support tool. The timing, findings and severities were summarised and compared to assess intra and inter pathologists variation.

Results

The results found that the use of Foresight increased study read speed on the Patholytix Study Browser by 21%. Aside from this improvement in speed, there were no other significant changes in performance metrics between the two reads. This suggests that while Foresight effectively enhances speed, it does not impact the accuracy, quality, or other aspects of performance in toxicology studies.

45: MULTIPLE AGE-RELATED DISEASES IN A CAPTIVE AFRICAN LION (PANTHERA LEO)

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Neoplasia, degenerative spine disease, and renal disease have been reported as causes of morbidity in captive geriatric lions. A 21-year-old male intact captive African lion (*Panthera leo*) was presented to Auburn University College of Veterinary Medicine Diagnostic necropsy services with a history of continuous weight loss and lethargy. Biochemistry revealed elevated liver and kidney enzymes. At necropsy, miliary, firm to hard, round to oval, raised, 3-mm to 3.5-cm in diameter, white nodules were scattered throughout the lung lobes. Multifocally, effacing and expanding 60% of the liver lobes were variably sized, up to 15 cm in diameter, uni-to multilocular to fluid-filled cysts, and pinpoint to up to 3 mm in diameter pale tan and flat foci. Within the affected liver, the cystic wall was green, dark green, or pale tan and the cystic fluid was thin, dark brown to dark green. The gallbladder was moderately distended with multiple cysts similar to those observed in the liver attached to the mucosa. Spanning throughout the entire spine, the vertebrae and intervertebral discs were severely affected by degenerative discs, bone protrusion, osteophytes, spondylosis, and osteosclerosis. Histopathologic evaluation of the lung lobes revealed pulmonary adenocarcinoma with neoplastic cells strongly immunolabeling for TTF-1 antigen. Biliary cysts and biliary cystadenoma were both observed in the affected liver. Severe and extensive papillary coagulative necrosis and chronic kidney disease presented in bilateral kidneys. These findings help document the pathologic findings in captive geriatric American lions and provide clinicians with information on disease differentials.