

64th AAVLD

Diagnostic Pathology Slide Seminar



American Association of Veterinary Laboratory Diagnosticians

Thursday, October 24, 2021, 3:30-6:00 PM

64th AAVLD Diagnostic Pathology Slide Seminar

Case	Submitter	Species	Diagnosis
1	Ford	Cottontail	Necrotizing hepatitis and splenitis (tularemia)
2	Lee	Tortoise	Intranuclear coccidiosis
3	Thomas	Dog	Pseudorabies
4	Lima	Horse	Intestinal mycobacteriosis
5	Pillai	Horse	Halicephalobus nephritis
6	Teodoro	Dog	Metastatic Digital Chondrogenic Melanocytic Tumor
7	Kleinhenz	Ox	Otitis
8	Clayton	Mink	SARS-CoV-2 pneumonia
9	Fingerhood	Cat	Pyogranulomatous intestinal leiomyositis and suspect vasculitis (<i>Bartonella henselae</i>)
10	Gaudette	Chicken	Air sac cystadenoma
11	Gruenwald	Cat	Feline progressive histiocytosis
12	Hill-Thimmesch	Pigeon	Necrotizing hepatitis (herpesvirus, adenovirus, circovirus)
13	Gonzalez-Viera	Sheep	Pyogranulomatous rhinitis and cellulitis (<i>Actinobacillus lignieresii</i>)
14	Streitenberger	Rabbit	Caliciviral hepatitis (RHDV2)
15	Rasche	Budgerigar	Beak leiomyosarcoma
16	Wong	Zebrafish	Microsporidial xenomas

Underlining denotes the presenting author.

Case 1

Necrotizing splenitis and hepatitis associated with *Francisella tularensis* infection in an Eastern cottontail rabbit (*Sylvilagus floridanus*)

A.K. Ford, M.A. Highland

Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, Kansas

Clinical history and gross findings: An adult, male, wild Eastern cottontail rabbit found dead from a reportedly large die-off was submitted for necropsy. Overall body condition was poor, and dozens of variably engorged ticks were firmly attached to the ears. Scant dried blood covered the fur surrounding the philtrum and mouth. The capsular surface of the spleen had numerous tan, flat to slightly raised, pinpoint foci that extended into, and were throughout, the splenic parenchyma. Similar foci were throughout all liver lobes.

Histopathology: Spleen: Coalescent areas of fibrin and eosinophilic and karyorrhectic debris (lytic necrosis) efface and replace >90% of the splenic architecture; lytic areas are occasionally surrounded by a few mixed inflammatory cells. Frequently throughout the section, often adjacent to vessels, are small-to-large sized colonies of gram-negative coccobacilli. Liver: Hepatic parenchyma is similarly affected by random, up to 400 µm diameter areas of lytic necrosis. Embedded within and surrounding necrotic foci are clusters of variably sized colonies of gram-negative coccobacilli.

Diagnoses: 1. Spleen: Splenitis, fibrinonecrotizing, diffuse, severe, with gram-negative coccobacilli; 2. Liver: Hepatitis, necrotizing, random, multifocal to coalescent, severe, with gram-negative coccobacilli

Liver: Hepatitis, necrotizing, random, multifocal to coalescent, severe, with gram-negative coccobacilli

Ancillary tests: Spleen submitted to the KSVDL microbiology laboratory isolated pure culture of suspect colonies of *Francisella tularensis*. Confirmatory culture was performed at the Centers for Disease Control (CDC).

Comments: Gross and histologic lesions are consistent with tularemia. Prior to necropsy, top differentials based on the large number of dead rabbits were tularemia and rabbit hemorrhagic disease virus (RHDV2). Tularemia is endemic in Eastern cottontails, and transmission occurs through arthropod bite, ingestion, inhalation, or direct inoculation. Confirmatory testing is through isolation of *F. tularensis* from fresh tissue, aspirates, or swabs. Polymerase chain reaction (PCR), direct immunofluorescence assay (DFA), and immunohistochemical staining are all considered supportive diagnostics according to the CDC. Often, *F. tularensis* bacteria are not identified microscopically without immunohistochemistry, however the numerous gram-negative coccobacilli in the liver and spleen are interpreted to be *F. tularensis* in this case, supported by the pure culture result.

References:

-Delaney MA, Treuting PM, and Rothenburger JL. Lagomorpha. In: Terio KA, McAloose D, and St. Leger J. *Pathology of Wildlife and Zoo Animals*, Volume 1. San Diego (CA): Elsevier; 2018.

Case 2

Intranuclear coccidiosis in a red-footed tortoise (*Chelonoidis carbonarius*)

J. Lee, R. Bauer, J. Nevarez

Louisiana State University, Baton Rouge, Louisiana

Signalment: Red-footed tortoise (*Chelonoidis carbonarius*), male, 1.5-year-old.

Clinical history: The tortoise had lethargy, anorexia, dyspnea, obtundation, ocular and nasal discharge, and soft plastron, carapace, and skull. Hypoglycemia, hypoproteinemia, hyponatremia, hypochloremia, hyperkalemia, and hyperuricemia were noted on bloodwork. Euthanasia was elected.

Gross findings: There is minimal amount of visceral fat. The liver is diffusely pale tan to yellow.

Microscopic description:

Liver: Low to moderate numbers of granulocytes infiltrate the portal tracts associated with mild biliary hyperplasia, degeneration or necrosis of biliary epithelial cells or hepatocytes. Frequent bile ductular epithelial cells and hepatocytes have intranuclear apicomplexan protozoal gametes and schizonts. The gametes are 5-15 μm in diameter, round, and have eosinophilic granules. The schizonts have elongated, 2-3 μm long, basophilic merozoites arranged in a parallel or radiating pattern. There is marked diffuse hepatocellular lipid-type vacuolation.

Kidney: There is frequent degeneration, necrosis, and regeneration of tubular epithelial cells. The lumina often contain eosinophilic proteinaceous material, degenerate epithelial cells and granulocytes.

Occasionally, the tubular epithelial cells contain intranuclear protozoa similar to that described in the liver. There is mild multifocal interstitial infiltration of granulocytes.

Morphologic diagnosis:

Liver: Severe hepatic lipidosis, mild subacute multifocal granulocytic cholangiohepatitis with intranuclear coccidia
Kidney: Moderate subacute multifocal tubular degeneration and necrosis with intranuclear coccidia

Laboratory results: Kidney positive for intranuclear coccidiosis.

Comment: Based on the species and histologic features, intranuclear coccidiosis of Testudines (TINC) is the most likely diagnosis. TINC is an emerging disease in chelonians. The causative agent has not been assigned to a genus yet. It was not until recently that oocysts have been detected from a leopard tortoise, and fecal-oral transmission has been demonstrated. TINC causes systemic infection and non-specific clinical signs. In this case, intranuclear coccidia were noted in the liver, kidney, lung, thyroid, stomach, and harderian gland.

Reference:

-Hofmannová, Lada, et al. "Intranuclear coccidiosis in tortoises—discovery of its causative agent and transmission." *European journal of protistology* 67 (2019): 71-76.

Case 3

Pseudorabies Viral Encephalitis in Dogs Hunting Feral Swine in Ashley County Arkansas

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³USDA, APHIS, Sherwood, Arkansas

Clinical history: Five mixed-breed 1.5 to 5.0-year-old canines were used to hunt feral swine on 31 May 21 in Ashley County, Arkansas. Within 5 days, 4/5 dogs were foaming at the mouth, lethargic, and anorexic with intense facial pruritus, often leading to self-induced trauma (“mad itch”). By day 6 all 4 of these dogs were dead.

Gross findings: Brain: Multifocal meningeal vessels were mildly congested, but otherwise the cerebrum, cerebellum, and brainstem were unremarkable.

Microscopic description: Brain (cerebral cortex at the level of the telencephalon): Multifocally affecting <5% of the of the section are areas of hypercellularity (gliosis) in both the gray and white matter, composed of oligodendrocytes, microglia, and astrocytes, occasionally with abundant hypereosinophilic cytoplasm (gemistocytes). Occasionally these cells localize around neurons (satellitosis) that are rarely shrunken, angular, and hypereosinophilic with pyknotic nuclei (neuronal necrosis). Rarely necrotic neurons are phagocytized (neuronophagia) by microglia with a small amount of eosinophilic cytoplasm (gitter cells) or contain 2-4 µm round lightly eosinophilic intranuclear inclusion bodies that marginate the adjacent chromatin (herpetic inclusions). There are scattered areas of jagged, coalescing clear space in the adjacent neuroparenchyma (rarefaction). Multifocal vessels are lined by plump reactive endothelium, with a mononuclear infiltrate composed lymphocytes and plasma cells forming an incomplete 1 cell layer thick cuff within the adjacent Virchow-Robin space.

Ancillary tests: Suid alphaherpesvirus 1 (SuHV-1) PCR test was positive on brain homogenate with a Ct of 27. SuHV-1 was isolated in PK-15 cells. Rabies virus direct fluorescent antibody testing on brain was negative.

Morphologic diagnosis: Encephalitis, necrotizing and lymphoplasmacytic, multifocal, with rare intranuclear inclusion bodies.

Comments: Serial sections of the brain were evaluated, and histologic lesions were inconsistently present in the cerebral cortex, cerebellum, and brainstem. Intranuclear inclusion bodies were rarely present in neurons. Inclusion bodies were not definitively identified in a similar case from 2014.¹ This suggests that viral inclusion bodies are not a consistent feature of SuHV-1 canine encephalitis, and their absence should not dissuade a diagnosis.

References:

-Pedersen, K et al. (2018) BMC Vet Res. 2018;14(1):388.

Case 4

Intestinal mycobacteriosis in a mare

J. Menk P. Lima, K. Vyhnal, F. Del Piero

Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, Baton Rouge, Louisiana

Clinical history: A 21-year-old quarter horse mare had a 2-month history of significant weight loss, lethargy and diarrhea. Anuria and dehydration were reported 2 days before euthanasia.

Gross findings: There was marked transmural edema of the cecum and ventral large colon, which had reddened and friable mucosa. The ileocecal lymph nodes were markedly enlarged.

Microscopic description: Colon and cecum. The lamina propria and submucosa are infiltrated and expanded by numerous macrophages and Langhans-type multinucleated giant cells, accompanied by plasma cells (frequent Mott cells), lymphocytes and eosinophils. Macrophages and multinucleated giant cells contain myriad, ill-defined bacteria that are negative for stain uptake with hematoxylin and eosin but stain bright red with an acid-fast (fites) stain. The submucosa is markedly expanded by edema, and lymphatic vessels are markedly ectatic.

Morphologic diagnosis: Colon and cecum: Typhlocolitis, granulomatous, multifocal, marked, with intracytoplasmic acid-fast bacilli, and severe submucosal and mucosal edema.

Ancillary tests: A polymerase chain reaction (PCR) on formalin-fixed samples of the colon was negative for *Mycobacterium tuberculosis* (IS 6110) and *Mycobacterium avium* (16S rDNA), and positive for *Mycobacterium avium* subspecies *paratuberculosis* (IS900 and MAP02).

Comments: The history, gross, microscopic and molecular findings are strongly suggestive of paratuberculosis in this mare. Spontaneous paratuberculosis has not been reported in horses to the authors' knowledge. While mycobacterial infections can cause chronic, progressive intestinal disease in horses, *M. avium ssp. avium* and *hominissuis* are the most commonly implicated agents. In this case, the identification of acid-fast bacilli within intralésional macrophages, and PCR detection of *M. avium subsp. paratuberculosis* with genes IS900 and MAP02 is supportive of paratuberculosis. Further identification of the F57 gene via PCR is still pending. The evidence gathered thus far suggests this is a potential first report of naturally-occurring paratuberculosis in an equine.

References:

- Fecteau ME. Paratuberculosis in cattle. Vet Clin North Am Food Anim Pract. 2018;34:209–222.
- Monki J, Hewetson M. Clinical Perspective: Intestinal Mycobacteriosis - A Rare Cause of Chronic Wasting Disease in Horses. J Mycobac Dis. 2014, 4:2.

Case 5

Halicephalobus gingivalis infection in a horse

V. Vijayan Pillai

Purdue University, West Lafayette, Indiana

Clinical history: A 19-year-old American Saddle horse gelding developed progressive ataxia and right circling. The animal was euthanized and necropsied.

Gross findings: Kidneys were enlarged with variably sized, pale tan, elevated masses expanding the cortical surface. On cut section, these masses effaced the parenchyma and multifocally expanded into the medulla. Masses of varying sizes were also observed on the right atrium, liver, spleen and adrenal glands.

Microscopic description: The renal interstitium was almost completely expanded and effaced by granulomas with numerous epithelioid macrophages, multinucleated giant cells, plasma cells, eosinophils, and few lymphocytes surrounded by dense bands of fibrous connective tissue and centered on numerous tangential and cross-sections of well-preserved roundworms (nematodes). The interstitium was diffusely expanded by previously described inflammatory cells. The remaining parenchyma showed diffuse atrophy and loss of glomeruli and tubules and shrunken, fibrotic glomerular tufts. Remnant renal tubules were multifocally moderately dilated and contained eosinophilic proteinaceous casts with numerous rhabditiform nematode parasitic larva, adults, and embryonated ova. The adult nematodes had a thin eosinophilic cuticle; a cylindrical body (13-20 μm in diameter) with tapered ends; rhabditiform esophagus with a characteristic corpus, isthmus, and bulb; and dorsoventrally reflected ovary. Larval nematodes were smaller (approximately 6-10 μm in diameter) and had a characteristic rhabditiform esophagus.

Morphologic diagnosis: Kidney: Nephritis, granulomatous, diffuse, chronic, with numerous intralesional nematodes

Comments: *Halicephalobus gingivalis* is a ubiquitous, free-living saprophytic nematode that has sporadically been associated with opportunistic infections in horses, zebras, cows, and humans. Information on the infection, pathogenesis, and life cycle of this nematode is limited. With the rarity of the disease, non-specific clinical and laboratory findings, and lack of availability of screening tests, a definitive diagnosis can be challenging in the absence of accessible lesions for biopsy. Confirmatory diagnosis is based on histopathologic demonstration of granulomas or granulomatous inflammation with characteristic intralesional nematode parasites with a tapered anterior head end, pointed tail, rhabditiform esophagus with distinct corpus, isthmus, and bulb, dorsoflexed ovary, and a ventroflexed uterus.

Case 6

Metastatic Digital Chondrogenic Melanocytic Tumor in a Dog

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¹Federal University of Lavras, Lavras, Brazil, ²North Carolina State University, Raleigh, North Carolina,

³Sao Paulo State University, Sao Paulo, Brazil

Clinical history: A 9-year-old, intact male, Brazilian Mastiff dog had a 1-year history of severe swelling in all four limbs with locomotory impairment. The dog was euthanized and submitted for autopsy.

Gross findings: Replacing the left hind limb's distal phalanx of the 5th digit was a firm, multilobulated, non-ulcerated, 3 cm diameter, black with randomly distributed white foci and striations cutaneous nodule. The appendicular skeleton, lungs, heart, pleura, mesentery, adrenal glands, and kidneys were infiltrated by coalescing similar nodules ranging from 0.1 to 3.5 cm in diameter.

Microscopic description: The masses were composed of polygonal to fusiform neoplastic cells arranged in short, interwoven parallel bundles and streams, eosinophilic cytoplasm with variable amounts of dark-brown granules, and oval to elongate nuclei with finely stippled chromatin. Forty-six mitotic figures were observed in ten hpf. Multifocal necrosis, myxoid matrix, and cartilaginous differentiation were present. The cellular cartilaginous component had moderate anisocytosis and anisokaryosis.

Ancillary tests: Neoplastic cells had strong positive cytoplasmic immunoreactivity for S100, with only 10% displaying a moderate cytoplasmic immunoreactivity for Melan-A.

Morphologic diagnosis: Lung: metastatic chondrogenic melanocytic tumor (metastasized from the distal phalanx of the left hindlimb 5th digit

Comments: Based on the gross, microscopic, and immunohistochemical findings, the diagnosis of metastatic digital chondroblastic melanocytic tumor was made. The potential of myoepithelial cells to differentiate into osteoblasts or chondroblasts is well known in canine mixed type mammary tumors and apocrine gland tumors. Similarly, the potential of melanocytic tumors to produce myxoid, chondroid, and/or osteoid matrix may be associated with the inherent capacity of the neural crest cells. The chondroid differentiation in melanocytic tumors can occur at any anatomical location but is seldom reported in dogs.

References:

-Sanchez J, Ramirez GA, Buendia AJ, et al. Immunohistochemical characterization and evaluation of prognostic factors in canine oral melanomas with osteocartilaginous differentiation. Vet Pathol 2007;44:676-682

-Smedley RC, Spangler WL, Esplin DG, et al. Prognostic markers for canine melanocytic neoplasms: A Comparative review of the literature and goals for future investigation. Vet Pathol 2011;48:54-72

Case 7

Otitis in a 1-month-old beef calf

K.E. Kleinhenz, B.L. Njaa, J. Henningson

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Clinical history: A 1-month-old crossbred beef calf developed a “droopy” head and died following a brief course of antimicrobial therapy (oxytetracycline and sulfamethazine).

Gross findings: Bilaterally, external auditory meatuses contained a moderate amount of red to gray, thick exudate that was also found in the middle ear. The right and left cranial lung lobes were diffusely dark red to purple with a sharp line of cranioventral consolidation. Numerous airways were ectatic with intraluminal, tan, caseous exudate. Interlobular septa were frequently expanded by mild to moderate amounts of edema. The liver had multiple tan, slightly depressed foci up to 2 mm in diameter throughout all lobes.

Microscopic description: The tympanic cavity, external acoustic meatus, sacculus, utriculus, cochlea, and auditory tubule contain few to abundant predominantly degenerate neutrophils admixed with macrophages, fewer lymphocytes, fibrin and proteinaceous material. The tympanic membrane is moderately expanded by edema, fibrin, degenerate neutrophils, and macrophages. The mucoperiosteum of the middle ear is moderately thickened by edema with simple squamous to cuboidal to columnar epithelium multifocally forming folds and invaginations. Irregularly scalloped edges with numerous reversal lines are observed in the stapes, septa of the tympanic bulla, and petrous portion of the temporal bone.

Ancillary testing: PCR was positive for *Histophilus somni* (Ct 20.60), *Mycoplasma bovis* (Ct 38.22), and *Pasteurella multocida* (Ct 35.08).

Morphologic diagnosis: Ear: Otitis externa, media, and interna, suppurative and histiocytic, diffuse, moderate with myringitis and bony remodeling

Comments: The pathogenesis of bovine otitis media and interna (OMI) remains to be fully understood. The current theory of OMI pathogenesis suggests the following mechanisms: hematogenous, retrograding infection via the auditory tube, or rupture of the tympanic membrane. OMI lesions are frequently reported sequela to bovine respiratory disease associated with the following bacterial agents: *Histophilus somni*, *Pasteurella multocida*, *Mycoplasma bovis*, *Streptococcus* spp., *Actinomyces* spp., and *Trueperella pyogenes*.

References:

- Njaa BL, Wilcock BP. Special Senses. In: Maxie MG, ed. Jubb, Kennedy and Palmer’s Pathology of Domestic Animals. Vol 1. 6th ed. 2016:496-498.
- Duarte ER, Hamdan JS. Otitis in Cattle, an Aetiological Review. J Vet Med B Infect Dis Vet Public Health. 2004 Feb;51(1):1-7.

Case 8

Outbreak of SARS-CoV-2 infection in farmed mink in Utah.

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Animal Dairy and Veterinary Science, Utah State University, Logan, Utah

Clinical history: In August of 2020, five mink farms in Utah experienced high mortality rates in adult mink. Mink kits were largely unaffected.

Gross findings: Lung lobes were diffusely dark red, failed to collapse, and oozed abundant red fluid when incised.

Microscopic description: In sections of lung, abundant edema fluid, moderate numbers of lymphocytes, few plasma cells, and rare neutrophils separate collagen fibers of the tunica adventitia of multiple vessels. Increased numbers of macrophages are in many alveolar spaces, accompanied by individual neutrophils. In scattered alveoli, fibrin strands overlie type I pneumocytes, which are infrequently necrotic. Type II pneumocytes sporadically line adjacent alveolar septa (not present in all slides). Small numbers of neutrophils and lymphocytes are in few alveolar septal capillaries multifocally. Edema fluid widens infrequent alveolar septa. Alveolar capillaries were diffusely congested.

Ancillary Test: SARS-CoV-2 was detected by real time RT-PCR in lung samples from all mink tested.

Morphologic diagnosis: Interstitial pneumonia and perivascularitis, lymphocytic, histiocytic and neutrophilic, diffuse, mild to moderate, acute, with congestion and edema.

Comments: SARS-CoV-2 was first diagnosed in farmed mink in the Netherlands in April of 2020 followed by reports in several other European countries. In the USA, the first outbreak occurred on five mink farms in Utah in August 2020. Subsequent SARS-CoV-2 infection in mink occurred in other states including Oregon, Michigan, and Wisconsin. Utah mink infected with SARS-CoV-2 exhibited severe respiratory disease with mortality ranging from 35% to 55% of adult mink. In Europe, only a 1.8 % increase in mortality occurred. Microscopic lesions differed as well. Interstitial pneumonia with thickened alveolar septa, hyaline membranes, and marked type II pneumocyte hyperplasia was reported in Dutch mink, whereas perivascularitis, congestion and edema were observed in mink in Utah. The lesions in mink in Utah indicate a more severe and acute course of the disease with fatal pulmonary edema.

References:

-Oreshkova N, Molenaar RJ, Et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Eurosurveillance*, 25, 2001005 (2020)

-Eckstrand C, Baldwin T, et al., An outbreak of SARS-CoV-2 with high mortality in mink (*Neovison vison*) on multiple Utah farms. Submitted for publication. *bioRxiv*.

Case 9

Pyogranulomatous intestinal leiomyositis and suspect vasculitis associated with *Bartonella henselae* in a kitten

S. Fingerhood, A. Choi

University of California, Davis, Davis, California

Clinical history: A 2-month-old, domestic shorthair cat presented for respiratory distress. Examination revealed tri-cavitary effusion, consistent with a modified transudate. Bloodwork revealed an inflammatory leukogram with a left shift, toxic change, and a moderate, non-regenerative anemia.

Gross Findings: Within the abdomen is 5 ml of slightly viscous, translucent, yellow, fluid admixed with scant strands of fibrin. Scattered across the serosa of the intestines are hundreds of 1 to 2mm diameter, firm, pale tan nodules.

Histopathology: The muscular layers of the jejunum, duodenum and stomach are disrupted by nodular accumulations of neutrophils, histiocytes, fibrin, and hemorrhage (suspect vasculitis). Admixed with the inflammation are densely packed, small vessel profiles with plump endothelia. Inflammation minimally extends into the submucosa and surrounding mesenteric fat. Pyogranulomatous inflammation multifocally disrupts the pancreatic parenchyma. Morphologic diagnoses: Pyogranulomatous leiomyositis, pancreatitis and suspect vasculitis with vascular proliferation, multifocal, marked

Ancillary testing: Feline coronavirus IHC & PCR and toxoplasma IHC (intestines): negative. *Bartonella henselae* qPCR (frozen colon): positive. *B. henselae* indirect immunofluorescence (FFPE intestines): positive, with organisms identified in regions of inflammation.

Comments: This case provides molecular and immunofluorescent evidence of *Bartonella henselae* infection in a kitten associated with systemic inflammation and tropism for smooth muscle. *Bartonella* species are vector-borne, fastidious, gram-negative bacteria that cause intra-erythrocytic and vascular-endothelial infections with relapsing bacteremia. Lesions in cats include endocarditis, myocarditis, and systemic reactive angioendotheliomatosis. This case uniquely demonstrates a prominent leiomyositis affecting the smooth muscle of the gastrointestinal tract. The vascular proliferation and muscle tropism provide a striking example of *Bartonella* infection that could easily be mistaken for feline infectious peritonitis.

References:

- Varanat M, et al. Identification of *Bartonella henselae* in 2 Cats With Pyogranulomatous Myocarditis and Diaphragmatic Myositis. *Veterinary Pathology*. 2012;49(4):608-611.
- Beerlage C, et al. *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* as potential causes of proliferative vascular diseases in animals. *Med Microbiol Immunol*. 2012; 201(3): 319–326.

Case 10

Air sac cystadenoma in a pet/hobby chicken

C. Gaudette, J. Nagel, C. Ellington, O. Fletcher, J. Barnes, L. Borst, R. Crespo, T. Terumi Negrao Watanabe
North Carolina State University, Raleigh, North Carolina

Clinical history: A 2-year-old, pet/hobby laying hen from a 60-bird flock had a 3-week history of wet snickering and wheezing. Other birds in the flock were not affected. The chicken was euthanized and submitted for autopsy.

Gross findings: At the level of the caudoventral neck, cranially to the left clavicle, was an extracorporeal outpouching structure representing an extension of the left cervical air sac. The wall of the cranial aspect of the affected air sac had a 2.0 x 0.9 x 0.8 cm, intraluminal, smooth, soft, raised, pale brown to light red mass. On cut surface, the mass had coalescing cysts ranging from 0.1 to 0.4 cm in diameter containing scant, light red fluid and a focal, pale tan to yellow, granular, friable, centrally located 0.2 cm diameter nodule.

Microscopic description: Focally expanding the left cervical air sac was a non-encapsulated, well-demarcated, moderately cellular neoplasm composed of cuboidal cells arranged in variably sized cysts lined by a single layer of cells. Neoplastic cells had indistinct cell borders, scant eosinophilic cytoplasm, and round to oval nuclei with finely stippled chromatin containing one nucleolus. Anisocytosis and anisokaryosis were mild; one mitotic figure was observed in 10 hpf. The mass was centrally replaced by numerous cholesterol clefts. Few scattered macrophages and lymphocytes are within the neoplasm.

Ancillary test: Approximately 10% of the neoplastic cells exhibited variable weak to strong cytoplasmic immunoreactivity for pancytokeratin.

Morphologic diagnosis: Left clavicular air sac: Air sac cystadenoma

Comments: Diagnosis of air sac cystadenoma was confirmed by histology and immunohistochemistry. Primary air sac neoplasms are uncommonly reported and when reported are often considered malignant with poor prognosis. Cystadenocarcinomas affecting the air sacs located near the axilla or humerus (clavicular air sac) have been reported. In this case, the neoplasm appeared to be associated with the clavicular or cervical air sac. Differential diagnosis should include thyroid and thymus neoplasms and any inflammatory/infectious process. To our knowledge, primary benign air sac neoplasms, as observed in this case, have not been previously reported in poultry.

References:

- Lightfoot TL, Garner MM. Chapter:20 Overview of tumors. In: Harrison GJ, Lightfoot TL eds. Clinical Avian Medicine. Palm Beach, FL: Spix Pub. 2006;2:559-571
- Raidal SR, et al. Airsac Cystadenocarcinomas in cockatoos. Aust. Vet. J. 2006 84:213-216

Case 11

End stage feline progressive histiocytosis with extensive visceral metastasis

R. Gruenwald, M. Kiupel, D.G. Sledge

Veterinary Diagnostic Laboratory, Michigan State University, Lansing, Michigan

History: This 7-year-old spayed female domestic shorthair cat was treated for skin cancer over the past year and recently became aphagic, adipsic, and nonambulatory. The animal died during hospitalization.

Gross Findings: Across all portions of the body including the head and distal limbs, there were dozens of raised, hairless, and often ulcerated white to tan skin nodules ranging in size from 5 mm to 5 cm in maximum diameter. There were similar firm, tan to white, 1-4 mm in diameter nodules in the lung and renal cortices. The liver was firm and mottled tan and red.

Histopathologic Findings: Cutaneous masses consisted of variably demarcated, expansile, dense sheets of neoplastic round cells effacing the dermis. Neoplastic cells had moderate amounts of pale eosinophilic cytoplasm, variably distinct borders, and round to oval, often centrally located, finely stippled nuclei with 1-2 variably prominent nucleoli. Anisokaryosis and anisocytosis were marked. Karyomegaly and multinucleated cells were common. There were up to 6 mitoses in 10 hpf. The epidermis was often ulcerated and replaced by thick serocellular crusts. Similar neoplastic cells formed peri-portal to random nodules within the liver, effaced portions of the renal cortex, and thickened alveolar septa of the lung.

Ancillary Findings: Neoplastic cells had strong membranous immunoreactivity for CD204 and were negative for CD3 and CD20.

Diagnosis: Feline Progressive Histiocytosis

Comments: Feline progressive histiocytosis (FPH) is an infrequently reported dermal histiocytic neoplasm of older cats. Initially, FPH presents as an indolent cutaneous disease with a predilection for the feet and head. In early lesions, neoplastic lesions appear histologically benign. In the terminal stages of disease, neoplastic cells display histologic features consistent with histiocytic sarcoma (HS), spread to draining lymph nodes, and metastasize to internal organs. While primary HS has been reported in cats, it is uncertain whether HS cases represent the end stage of FPH. Using IHC, immunophenotypical variants of feline histiocytic neoplasms have been described, which may indicate that FPH represents a heterogeneous group of neoplasms. In this case, IHC for CD204 was used to confirm the histiocytic origin of the neoplasm. As the neoplasm primarily affected the skin, including the head and limbs, a diagnosis of an advanced stage of FPH with subsequent metastasis was made.

References:

- Affolter VK, Moore PF. Feline Progressive Histiocytosis. *Veterinary Pathology*. 2006;43(5):646-655.
- Moore PF. A review of histiocytic diseases of dogs and cats. *Vet Pathol*. 2014 Jan;51(1):167-84.

Case 12

Multiple viral agents in racing pigeons

Kristen J Hill-Thimmesch; Tsang Long Lin, Geoffrey Lossie
Purdue University, West Lafayette, Illinois

Clinical history: Three adult racing pigeons from a 3000 bird flock were submitted following increased mortality and suspected pigeon circovirus (PiCV).

Gross findings: All pigeons were mildly emaciated with renal gout.

Microscopic description: The hepatic architecture was disrupted by multifocal to coalescing areas of hepatocellular degeneration and necrosis. Moderate numbers of hepatocytes exhibited karyomegaly with round, deeply basophilic, intranuclear inclusion bodies 5-8 micrometers in diameter. Few hepatocytes contained round, eosinophilic to amphophilic intranuclear inclusion bodies, 2-5 micrometers in diameter, surrounded by a clear halo that peripheralized chromatin. Numerous macrophages, lymphocytes, plasma cells, and few heterophils infiltrated hepatocellular degeneration and necrosis, cuffed vessels, and infiltrated few vessel walls.

Ancillary tests: PiCV and pigeon herpesvirus 1 (CoHV1) were detected from samples of liver submitted to the Utah Veterinary Diagnostic Laboratory. EM negative staining of liver revealed adenoviridae-like particles. A cloacal swab submitted to NVSL detected pigeon paramyxovirus type 1 (PPMV1).

Morphologic diagnosis: Lymphohistiocytic necrotizing hepatitis with basophilic and eosinophilic intranuclear viral inclusions

Comments: The hepatic lesions in conjunction with EM and PCR results were suggestive of adenovirus and CoHV1. Pigeon adenoviruses are organized into type 1 "classical adenovirus" and type 2 "necrotizing hepatitis". Type 1 occurs in juvenile pigeons and results in high morbidity, diarrhea, vomiting, and weight loss. Type 2 manifests as sudden death of adult pigeons, 30-100% mortality, and hepatic necrosis with viral inclusions. CoHV1 causes respiratory infections and published cases describe hepatic necrosis and viral inclusions. Adenovirus, CoHV1, PiCV, and PPMV 1 were detected by histologic, molecular, and EM techniques. Multiple histopathologic lesions suggestive of these viral agents were observed. These included lymphoplasmacytic necrotizing pancreatitis suggestive of PPMV1, cloacal bursal inflammation with atrophy and botryoid inclusions suggestive of PiCV, and intestinal intranuclear inclusions supportive of adenovirus.

References:

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Case 13

Pyogranulomatous rhinitis and facial cellulitis associated with *Actinobacillus lignieresii* in a ewe

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Clinical history: A 127 lbs., adult, mixed-breed ewe had severe facial and perioral swelling, rostral alopecia, respiratory distress, and dysphagia. The ewe was euthanized due to the poor prognosis.

Gross findings: The right cheek, the muzzle, and the right upper and lower lips are swollen, alopecic, light pink to white, and the subcutis is fibrotic. Over the right cheek, there are approximately 10 ulcers. The nasal turbinates and cribriform plate are thickened and contain numerous, mildly raised, pale-yellow, 0.5 cm nodules. A film of mucoid exudate covers the turbinates. The retropharyngeal lymph nodes are markedly enlarged. Miliary gray-tinged nodules are scattered throughout the lungs.

Histopathology: The nasal turbinates are diffusely obliterated by coalescing pyogranulomas often with central Splendore-Hoeppli material and numerous coccobacilli. There is spindle cell proliferation with fibrous connective tissue around the inflammatory foci. The surface epithelium has neutrophilic exocytosis. The lumen contains mucus, erythrocytes, neutrophils, sloughed epithelium, and large lakes of neutrophils with some histiocytes.

Morphologic diagnosis: Severe multifocal to coalescing chronic pyogranulomatous rhinitis with Splendore-Hoeppli and intralesional coccobacilli

Ancillary testing: Gram stain: The intralesional bacteria were gram-negative. Aerobic culture swabs of the nodules in the turbinates grew *Actinobacillus lignieresii* and *Pasteurella multocida* type A.

Comments: The pathologic and bacteriologic assessment confirmed the diagnosis of *A. lignieresii*-associated pyogranulomatous rhinitis. Other findings were pyogranulomatous facial cellulitis, cheilitis, and bronchopneumonia. *A. lignieresii* is part of the normal microbiota of the upper gastrointestinal tract and causes disease when it gains access to adjacent soft tissue via penetrating wounds. In cattle, the tongue is the most common site of infection. In sheep, it may cause sporadic outbreaks without the involvement of the tongue. However, the subcutaneous tissue of the head and nasal turbinates are more commonly affected. *P. multocida* type A associated with the rhinitis is one of the most prevalent and virulent types due to the hyaluronic acid in the capsule that damages the nasal mucosa. *P. multocida* type A likely exacerbated the actinobacillosis in this ewe. *A. lignieresii*-associated rhinitis and cellulitis in sheep is not frequently reported in peer-review literature.

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Case 14

Severe acute hepatic necrosis caused by rabbit hemorrhagic disease virus 2 (RHDV2) in a domestic rabbit (*Oryctolagus cuniculus*)

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Clinical history: The carcass of a 6-mo, male, standard Rex rabbit from a backyard rabbitry in southern California with history of 40.9% mortality during 3 days was submitted for postmortem examination and diagnostic workup.

Gross findings: The carcass was in good nutritional condition. Lungs were diffusely swollen and wet with multifocal dark-red areas in all pulmonary lobes. The trachea contained scant and stable foam, and the mucosa was diffusely dark red. The liver was friable, enlarged with an enhanced reticular pattern, and had faint areas of pallor. Mild splenomegaly was also noted.

Microscopic description: There are multiple periportal to midzonal and bridging areas of hepatocellular hypereosinophilia, dissociation, fragmentation, pyknosis, and karyorrhexis (necrosis). These areas are admixed with fibrin, heterophils, macrophages, and nuclear and cellular debris. There are occasional hepatocytes with swollen and vacuolated cytoplasm (vacuolar degeneration, lipid type). Multifocally, portal tracts are mildly infiltrated by lymphoplasmacytic and histiocytic cellular infiltrates.

Morphologic diagnosis: Severe, acute, multifocal, periportal to midzonal hepatocellular necrosis.

Ancillary tests: RHDV2 RT-qPCR was positive (Ct 13.67) in the liver. Pan-lagovirus IHC revealed positive signal associated with the areas of necrosis.

Comments: RHDV2 is an enveloped, single-stranded, positive-sense RNA virus (family *Caliciviridae*, genus *Lagovirus*). This virus is extremely contagious and affects kits and adult lagomorph species including domestic rabbits, cottontail rabbits, and hares and jackrabbits. The current USA outbreak is broadening its host range by exposing lagomorph species that are native to the American continent and the virus had not encountered before. The incubation period is 3-9 days; mortality can reach up to 70% - 100% depending on the strain. RHDV2 replicates in the hepatocytes causing hepatocellular damage and disseminated intravascular coagulation. Gross lesions include: blood in and around body orifices, reticular pattern in the liver, splenomegaly, and congestion and hemorrhages in several organs. Microscopic lesions are periportal to panlobular hepatic necrosis, lymphoid depletion, splenic necrosis, and fibrin microthrombi in multiple locations such as the glomerular and pulmonary capillaries. Differential diagnoses include septicemic *Pasteurella multocida*, tularemia, Tyzzer's disease, and anticoagulant rodenticide toxicosis.

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Case 15

Beak leiomyosarcoma in a budgerigar

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Clinical history: A 2-year-old male budgerigar died after a 1-day history of fracture of the rostral rhinotheca with pale mucous membranes, dyspnea, dull mentation, and ataxia.

Gross findings: The rostral tip of the rhinotheca was absent with jagged lateral edges covered with dried blood.

Microscopic description: Infiltrating the dermis and bone in the rostral upper beak was a poorly demarcated, non-encapsulated, infiltrative neoplasm composed of densely cellular interweaving streams of spindle cells. Neoplastic cells had a small amount of eosinophilic cytoplasm with indistinct cell borders and elongate nuclei with densely stippled chromatin. Anisocytosis and anisokaryosis were mild with 233 mitotic figures in 10 hpf. Multifocal necrosis was present in the neoplasm. Neoplastic infiltration of the bone was associated with osteolysis and osteonecrosis. The epidermis and cornified material along the ventral aspect of the beak tip was absent with exposure of the underlying neoplasm and abundant hemorrhage, necrotic debris, and mixed bacteria along the ulcerated surface. Neoplastic cells infiltrated a nerve and ganglion dorsal to the nasal cavity and an optic nerve.

Ancillary tests: Approximately 80% of the neoplastic cells exhibited strong cytoplasmic immunoreactivity for α -SMA but lacked immunoreactivity for S100, Melan-A, and PNL2.

Morphologic diagnosis: Upper beak: leiomyosarcoma with neural invasion, osteolysis, and rostral rhinotheca fracture

Comments: Based on the histologic appearance and location of this neoplasm, differential diagnoses included fibrosarcoma, peripheral nerve sheath tumor, amelanotic melanoma, and leiomyosarcoma. Fibrosarcomas are the most common primary beak tumor in budgerigars and are associated with pathologic beak fractures; therefore, this differential was initially prioritized. However, immunoreactivity for α -SMA supported a diagnosis of leiomyosarcoma. Leiomyosarcomas in birds are most frequently reported in visceral organs with local invasion and infrequent metastasis. Visceral metastasis was not observed in this case, but local invasion in nerves and a ganglion was present. To our knowledge, primary beak leiomyosarcoma has not been previously reported in birds.

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Case 16

Microsporidiosis in the spinal cord of a zebrafish (*Danio rerio*)

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Clinical history: An academic facility was interested in establishing a zebrafish health monitoring program for their research colony. Thirty live zebrafish of various strains were submitted for surveillance of background and underlying diseases.

Gross findings: No gross abnormalities.

Microscopic description: In a parasagittal section of a whole zebrafish, the neuropil of the myelencephalon and spinal cord are multifocally expanded by up to 100 um diameter, irregularly round microsporidian xenomas filled with 5 x 2.5 um, ovoid spores. Few xenomas are ruptured, and spores are surrounded by low numbers of macrophages and lymphocytes. Similar inflammation infiltrates spinal nerves, ganglia, and adjacent skeletal muscle.

Ancillary tests: Luna, Gram, acid-fast, and Giemsa stains variably highlight spores.

Morphologic diagnosis: Brain and spinal cord: Microsporidian xenomas (consistent with *Pseudoloma neurophilia*), severe, with mild lymphohistiocytic myelitis, ganglioneuritis, and myositis

Comments: Zebrafish are of increasing importance as a biomedical research model in studies ranging from aging, toxicology, oncology, behavior, neuroscience, immunology, and infectious disease. In a multi-institutional retrospective case series of over 10,000 zebrafish, *Pseudoloma neurophilia* was the most common pathogen/disease. Most cases are subclinical, however heavy infections can cause altered host immunity, inflammation, behavioral change, and skeletal deformities. Furthermore, there is a reported sex-specific impact in which infected female zebrafish have reduced body condition and fecundity. Therefore, it is critical to recognize this pathogen as a potential confounding factor in zebrafish research studies. A differential for xenomas in laboratory zebrafish is *Pleistophora hypohessobryconis*, which is found primarily in skeletal muscle.

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