Joint Pathology Center

Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2016-2017

C o n f e r e n c e 18

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CASE I: NF-15-1564 (JPC 4083683).

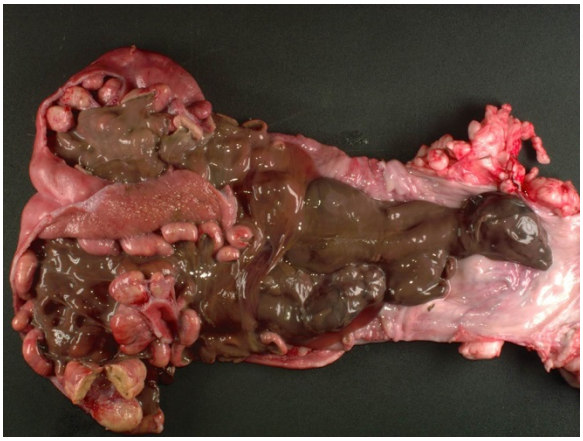
Signalment: Six-year-old female French Alpine goat, (*Capra aegagrus hircus*).

History: This goat was suspected to have aborted prior to necropsy, although no expelled fetus was found. This goat also aborted last year, but had successfully kidded in the past.

Gross Pathology: The uterine body contained two macerated fetuses with a crown to rump length of 16 cm and 10 cm. Within the uterus, all of the caruncles were enlarged (~4x2x1 cm), homogeneous, and pale tan.

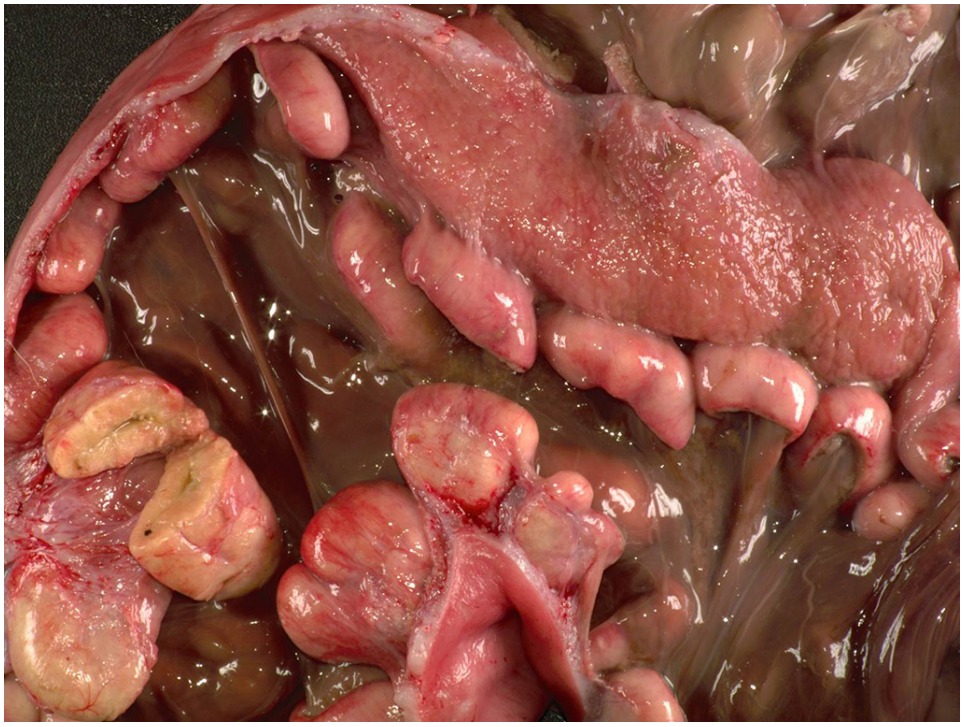
Laboratory results: Bacterial culture and sensitivity (uterus):
Numerous *Escherichia coli*
Few *Enterococcus faecalis*
No growth of *Brucella species*
Chlamydophila sp, PCR (uterus): Negative
Coxiella burnetii, PCR (uterus): Negative

Histopathologic Description: The uterine caruncular labyrinth is diffusely expanded by abundant pale eosinophilic homogenous, extracellular material which is multifocally disrupted by areas of blue granular mineralization. The interdigitating cotyledonary villi are sparse, and the allantoic stroma is mildly expanded by edema. The aforementioned interstitial eosinophilic material within the caruncles



Uterus, goat. The uterus contained two macerated feti. Caruncles were enlarged and pale tan. (Photo courtesy of: Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University. www.pathobiology.msu.edu)

stains orange/pink with Congo red and exhibits apple green birefringence with polarized light, consistent with amyloid. The umbilicated surface of the placentome is multifocally ulcerated and replaced by large aggregates of neutrophils, lymphocytes, and histiocytes. Similar inflammatory cells extend into the subepithelial stroma of the caruncle, endometrium, and minimally throughout the labyrinth. The placental and endometrial stroma is expanded by moderate amounts of edema, few scattered inflammatory cells, and multifocal aggregates of mineral. There are also multifocal areas of mineralization throughout the tunica media of medium-sized vessels within the placenta and endometrium.



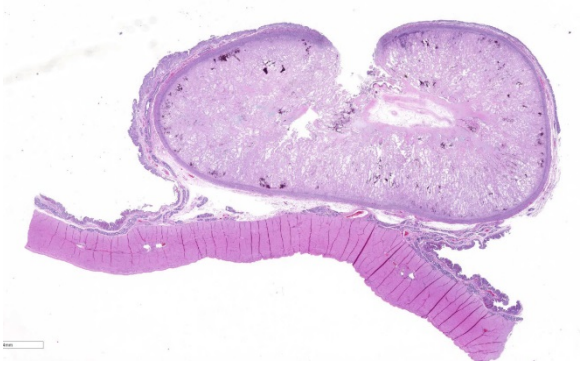
Uterus, goat. Closer view of caruncles, with one incised. (Photo courtesy of: Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University. www.pathobiology.msu.edu)

Contributor's Morphologic Diagnoses: 1. Uterus: Diffuse interstitial caruncular amyloid

2. Uterus and placenta: Chronic necrotizing placentitis and endometritis with mineralization

Contributor's Comment: Caruncular amyloidosis has been previously reported in a small number of goats in California. Clinical presentation of such goats included mid-to-late term abortion that often occurred repeatedly over multiple years which was attributed to impaired gas exchange at the site of fetal attachment.[2] Ages ranged from 3-8, and breeds included Toggenburg, La Mancha, and Saan. Similar to the California goats, this goat had no evidence of amyloid deposition in other organs nor was there evidence of a systemic or chronic disease process. Few bacteria were isolated from the inflamed region of the placentome but are considered to be secondary to the retained fetuses. Bacterial culture did not isolate *Brucella abortus*, and PCR was negative for *Chlamidophila species* and *Coxiella burnetti.*

In general, amyloid is composed of insoluble aggregates of misfolded proteins, and deposition of amyloid can occur in a wide variety of localized or systemic diseases.[8] Although the fibrillar component of amyloid is overall similar in composition, a diverse number of proteins with variation in sequence and structure are considered amyloidogenic.[7,8] Common amyloid precursors include: serum amyloid A (SAA),



Placentome, goat. At subgross magnification, the caruncle is diffusely pale and eosinophilic suggesting infarction (HE, 5X)

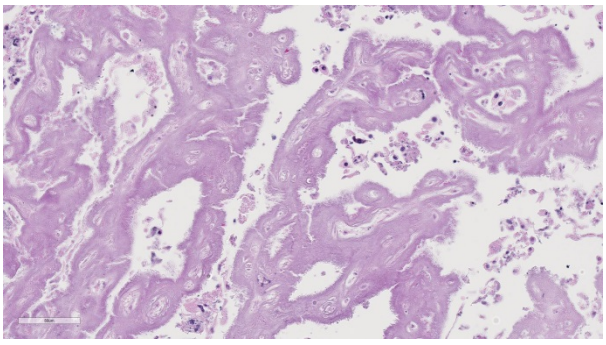
amyloid light chain (AL), islet amyloid polypeptide (IAPP), mutated forms of transthyretin, and beta protein amyloid.[7,8]

In particular, SAA proteins comprise a family of apolipoproteins that can be synthesized hepatically and/or extrahepatically. Hepatic derived SAA (SAA1 and SAA2) can dramatically increase in response to inflammation. In mice, rats, cows, and rabbits SAA3 appears to be the most common extrahepatic SAA in addition to being produced hepatically.[3] Increased production of SAA3 has also been described in bovine and human mammary gland epithelium in response to prolactin, and in uterine papillary cancer. In the goats in California increased levels of SAA3 were detected within the endometrium when compared to the liver, suggesting localized expression. The type and cause of the amyloid deposition in this case is currently unknown, but the localized caruncular involvement is similar to what has been previously described and may represent a new syndrome of goats.[2]

JPC Diagnosis: Placenta, caruncle: Amyloidosis, diffuse, marked, French Alpine goat, *Capra aegagrus hircus*.

Conference Comment: The contributor provides an informative summary of the pathogenesis of amyloidosis and review of previously reported cases of a unique syndrome of caruncular amyloidosis causing abortion in goats. This excellent case confounded conference participants on initial examination of the tissue section. Virtually every attendee interpreted the amorphous, smudgy, homogenous eosinophilic material that diffusely expands the uterine lattice as a geographic area of coagulative necrosis admixed with multifocal mineralization and mild inflammatory infiltrate in the subepithelial stroma of the caruncle and endometrium; collectively, the findings were attributed to normal post kidding involutional change, rather than caruncular amyloidosis. Similar to the findings reported by the contributor, Congo red histochemical staining of serial sections performed at the JPC demonstrates the eosinophilic proteinaceous material is diffusely congophilic and displays bright apple-green birefringence when viewed under polarized light.

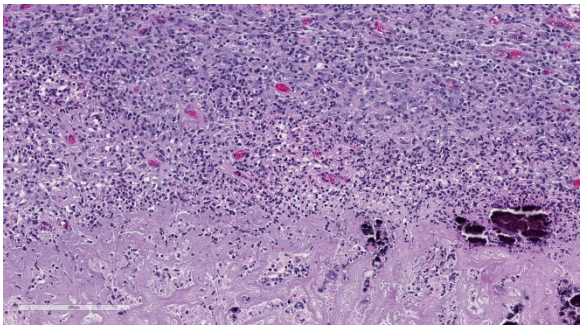
Spirited discussion ensued among conference participants regarding the presence of concurrent diffuse necrosis, autolysis, or a combination of both admixed with the deposited amyloid in the tissue section. Most favored diffuse necrosis of



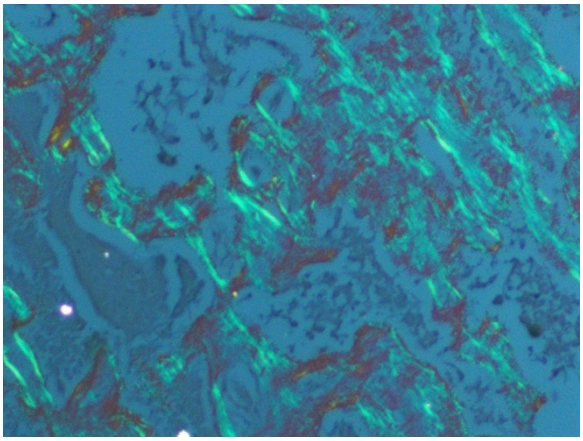
Placentome, goat. At high magnification, the caruncular labyrinth is diffusely eosinophilic and expanded with a loss of epithelium and there are small amounts of cellular debris in

both the epithelial and endothelial cells secondary to amyloid deposition, resulting in infarction of the placentome. Discord over the presence or absence of necrosis or autolysis nothwithstanding, this case nicely demonstrates a newly reported syndrome of reproductive failure in goats secondary to uterine amyloid deposition in the endometrium at the site of placental attachment.[2] Accumulation of amyloid within the carucle markedly compromises blood flow and both gas and nutrient exchange between the doe and the fetus; this leads to fetal hypoxia and eventually death.[2] Similar to previously reported cases of caruncular amyloidosis in goats[2], amyloid is not present within the intercaruncular endometrium, myometrium, endometrial glands, or vessels in this doe.

Before discussing this case, participants reviewed the normal placentation in small ruminants. All ruminants have similar cotyledonary placentation composed of the fetal cotyledon and the maternal caruncle. The placenta contains the maternal endometrium and the fetal chorioallantoic membranes (CAM).[1] Ruminant placentas are nondeciduate, indicating that the maternal endometrium and fetal CAM are in close contact, but they do not intimately fuse. In cotyledonary placentation, there are



Placentome, goat. At the periphery of the placentome, there is an infiltrate of moderate numbers of viable and degenerate neutrophils as well as multifocal mineralization. (HE, 172X)



Placentome, goat. Caruncular amyloid demonstrates green birefringence when viewed with polarized light. (Congo Red, 400X) (Photo courtesy of: Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University. www.pathobiology.msu.edu)

numerous areas where the fetal cotyledon villar attachments interdigitate with the crypts of the caruncular epithelium. The combination of the fetal cotyledon and maternal caruncle make up the placentome.[1] In sheep and goats, the caruncles have a characteristic concave shape, nicely demonstrated in this case.[1] Bovine placentomes are similar in structure and function, but are convex rather than concave.

Conference participants discussed various causes of abortion in small ruminants, to include infectious agents such as *Chlamydophila abortus*, *Toxoplasma gondii*, *Brucella ovis*, *Campylobacter fetus*, *Coxiella burnetii*, and *Listeria monocytogenes*. Non-infectious causes include plant toxins, such as locoweed poisoning, and nutritional factors including dietary deficiencies of copper, magnesium, vitamin A, and selenium.[5,6] After reviewing this case, conference participants agreed that caruncular amyloidosis should be considered as an additional differential diagnosis of non-infectious abortion in the goat.

Contributing Institution:
Department of Pathobiology and Diagnostic Investigation
College of Veterinary Medicine
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http://www.pathobiology.msu.edu

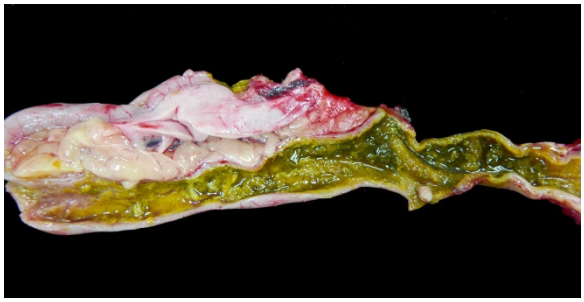
References:

1. Bacha WJ, Bacha LM. *Color Atlas of Veterinary Histology*. 3rd ed. Baltimore, MD: Lippincott Williams & Wilkins; 2012:243-260.
2. Gaffney PM, Barr B, Rowe JD, Bett C, Drygiannakis I, Giannitti F, Trejo M, Ghassemian M, Martin P, Masliah E, Sigurdson C. Protein profiling of isolated uterine AA Amyloidosis causing fetal death in goats. *FASEB J*. 2015; 29:911-919.
3. Larson MA, Wei SH, Weber A, Weber AT, McDonald TL. Induction of human mammary associated serum amyloid A3 expression by prolactin or lipopolysaccharide. *Biochem Biophys Res Commun*. 2003; 301:1030–1037.
4. O'Brien TD, Butler PC, Westermark P, Johnson KH. Islet amyloid polypeptide: A review of its biology and potential roles in the pathogenesis of diabetes mellitus. *Vet Pathol*. 1993; 30: 317-332.
5. Sanad YM, Jung K, Kashoma I, et al. Insights into potential pathogenesis mechanisms associated with *Campylobacter jejuni*-induced abortions in ewes. *BMC Vet Res*. 2014; 10:274-287.
6. Schlafer DH and Foster RA. Diseases of the gravid uterus, placenta and fetus In: Maxie MG, ed. *Jubb Kennedy and Palmer's Pathology of Domestic Animals*. Vol 3. 6th ed. Philadelphia, PA: Elsevier Saunders; 2016:407-408.
7. Tani Y, Uchida S, Nakamura H, Nakayama N, Goto D. Amyloid deposits in the gastrointestinal tract of aging dogs. *Vet Pathol*. 1997; 34:415-420.
8. Teoh SL, Griffin MD, Howlett GJ. Apolipoprotein and amyloid fibril formation in atherosclerosis. *Protein Cell*. 2011; 2(2):116-127.

CASE II: 58716 (JPC 4084216).

Signalment: 16-year-old, male, bar-headed goose, (*Anser indicus*).

History: Between September 9 and September 17, 2015, three bar-headed geese housed in an open, outdoor enclosure near Village Lagoon died unexpectedly. The first two had been found dead on exhibit, while the third had been hospitalized shortly before death with clinical signs of labored breathing and lethargy. The hospitalized goose had a CBC within normal limits. At the same time, a fourth bar-headed goose was hospitalized with diarrhea.



Intestine, bar-headed goose. The intestine is lined by a greenish yellow necrotic membrane. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global (WSC 77) http:/institute.sandiegozoo.org/wildlife-disease-laboratories)

Gross Pathology: The spleen has a few ill-defined, pale tan foci of necrosis extending from the serosa into the parenchyma. The pancreas has 10 to 15 scattered, tan, well-circumscribed necrotic foci measuring up to 0.2 cm in diameter. The entire small and large intestine are filled with tan to green, fibrous, clumped soft material that coats the mucosa in some regions and sloughs away along other portions. Within the ceca, there is a small to moderate amount of tan-green watery fluid and tan fibrous strands that loosely adhere to the mucosal surface.

Laboratory results: PCR and virus isolation were performed by the California Animal Health and Food Safety diagnostic laboratory (CAHFS).

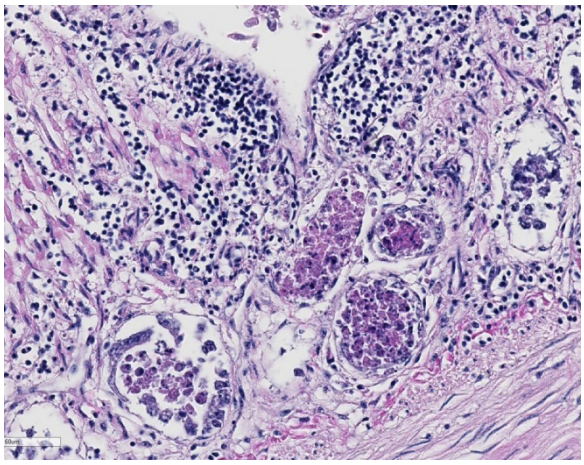
West Nile Virus (avian) PCR: Virus detected in brain

Virus isolation: West Nile Virus

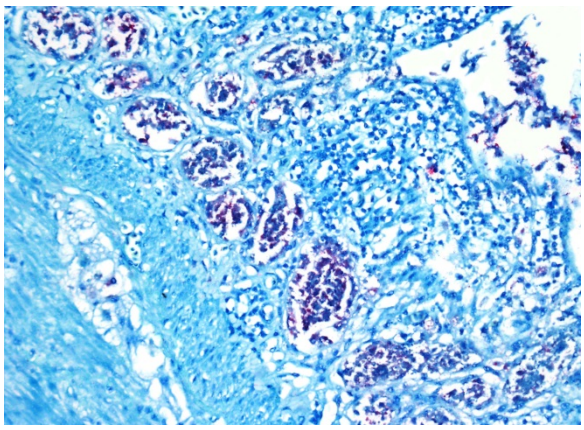
Avian Influenza Matrix Gene qRT-PCR: Not detected in lung, duodenum, or spleen.

Avian Paramyxovirus-1 qRT-PCR: Not detected in lung, duodenum, or spleen.

Bacterial culture, colon contents (IDEXX): 3+ *Escherichia coli*, 1+ *Aeromonas species*,



Intestine, bar-headed goose. There is marked necrosis of intestinal crypts. (HE, 324X).

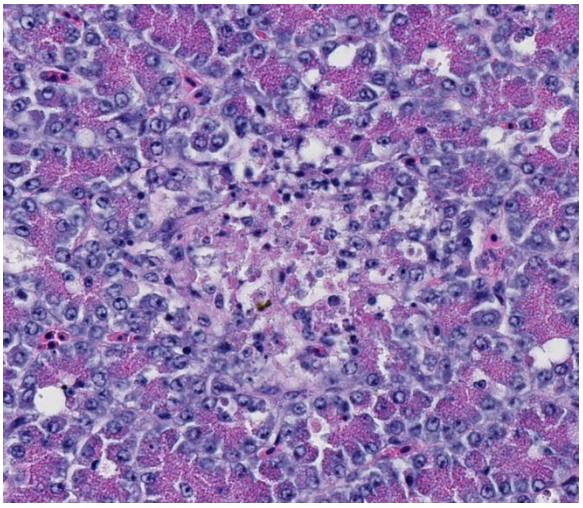


2+ Normal positive flora. No *Salmonella, Shigella, Pleisiomonas, Edwardseilla,*

Intestine, bar-headed goose. Intestinal epithelial cells within crypts exhibit strong cytoplasmic immunopositivity for West Nile virus. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global (WSC 77) http:/institute.sandiegozoo.org/wildlife-disease-laboratories)

Aeromonas or *Yersinia* were isolated.

Histopathologic Description: Duodenum and pancreas: Sections are mildly autolyzed. In the duodenum, villi are markedly blunted and frequently fragmented. The majority of crypts are dilated with hypereosinophilic, karyorrhectic cellular debris (crypt abscesses), and glandular epithelial cells are frequently shrunken and hypereosinophilic with nuclear pyknosis (necrotic). The villous epithelium is sloughed and is regionally replaced by luminal bands of necrotic sloughed cells, degenerate heterophils, and large numbers of embedded bacteria (pseudomembrane). Moderate to large numbers of lymphocytes with fewer plasma cells populate the lamina propria. Throughout the exocrine pancreas, there are multiple, randomly distributed foci of necrosis, characterized by hypereosinophilia, loss of cellular detail and karyorrhexis, and small numbers of heterophils. Foci of necrosis are often surrounded by a band of acinar cells with



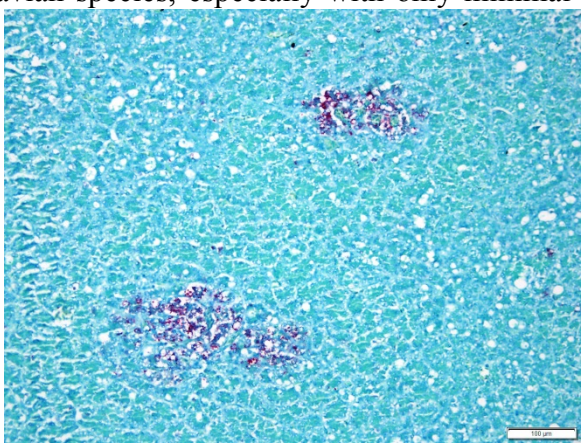
Pancreas, bar-headed goose. Foci of necrosis scattered randomly throughout the exocrine pancreas. (HE, 330X).

marked zymogen depletion. There are occasional ill-defined regions of exocrine pancreatic hyperplasia, with lobules of tightly packed, slightly haphazardly arranged acinar cells delineated by coarse fibrous septa.

Immunohistochemistry for West Nile Virus (performed in-house): Strong positive cytoplasmic immunoreactivity within intestinal epithelial cells or foci of crypt necrosis, pancreatic acinar cells, glial nodules in the brain, renal tubular epithelial cells, cardiomyocytes, hepatocytes, and in the spleen (macrophages).

Contributor's Morphologic Diagnoses: 1. Duodenum: Severe, diffuse, acute, necrotizing enteritis with segmental pseudo-membranes and mixed bacteria
2. Pancreas: Moderate, multifocal, acute necrosis

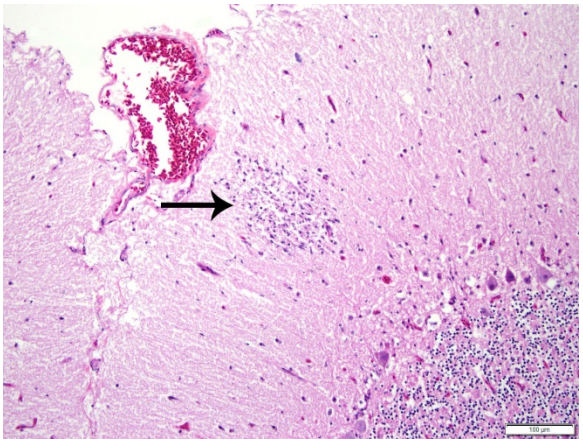
Contributor's Comment: Necropsy and histopathology findings on all three bar-headed geese (*Anser indicus*) were consistent with severe systemic viral infection, with acute necrosis most severely affecting the gastrointestinal tract, but also including pancreas, trachea, spleen, esophagus, feather follicle epithelium and brain. The three mortalities within a short time span, in conjunction with the spectrum of histologic lesions (widespread tissue necrosis), met internal criteria requiring notification of government regulatory officials (California Department of Food and Agriculture, CDFA) and testing to rule out highly-pathogenic avian influenza (HPAI). Immediate biosecurity measures were implemented. Suspicion of HPAI was heightened by the fact that bar-headed geese were the migratory waterfowl species predominantly affected in H5N1 HPAI virus epidemics in China in 2005 and 2006.[6,9] Within approximately 48 hours of notifying CDFA, we received negative test results for HPAI, and CDFA lifted the quarantine. Concurrently, West Nile virus (WNV) infection in all three geese was confirmed by in-house immunohistochemistry. These results were corroborated by PCR and virus isolation performed by the California Animal Health and Food Safety diagnostic laboratory (CAHFS). The presence of such striking, acute intestinal crypt necrosis was considered to be unusual for WNV in an avian species, especially with only minimal



Pancreas, bar-headed goose. WNV antigen is present within foci of pancreatic necrosis. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global (WSC 77) http:/institute.sandiegozoo.org/wildlife-disease-laboratories)

brain lesions and an absence of myocardial lesions.

West Nile Virus is in the genus *Flavivirus*, family *Flaviviridae*, and is serologically classified within the Japanese encephalitis antigenic group. The virus is distributed worldwide and has a wide potential host range, but is maintained primarily in a bird-mosquito cycle. Wild birds (especially corvids) act as amplifying hosts. *Culex spp* mosquitoes are the primary vector, although the virus is found in other vectors (other mosquito species, ticks) of undetermined significance in transmission.[3,5] Horizontal transmission[2] and transmission through prey or contaminated water have also been reported.[5] There are seven genetic lineages of WNV strains, with two major lineages, lineage 1 and lineage 2. WNV genetic lineage 1 is widespread geographically, including in North America. Lineage 2 WNV strains correspond primarily to enzootic areas in Africa, with recent detection in Europe. Lineage 1 strains have been considered more virulent, but both have been implicated in significant disease outbreaks in birds.[3,5] Mortality due to WNV has been documented in 24 orders of birds from North America, including anseriforms.[5]



Cerebellum, bar-headed goose. Glial nodules are present throughout the brain. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global (WSC 77) http:/institute.sandiegozoo.org/wildlife-disease-laboratories)

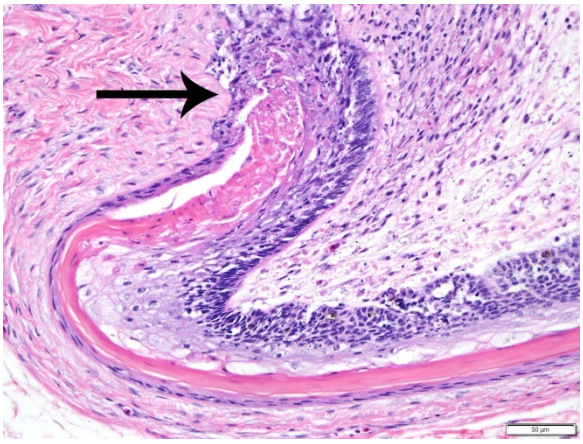
In mammals, after a bite by an infected mosquito, the virus replicates in keratinocytes, cutaneous dendritic cells, endothelial cells, and fibroblasts, followed by viremia and hematogenous spread.[3] In avian species, the mechanism and sites of replication are not completely understood. Experimentally in birds, virus has been detected in blood within 45 minutes of mosquito biting, suggesting that primary viremia can occur without local replication.[5] Clinical disease develops upon viral invasion of major organs and/or the CNS, usually by 5-6 days post-infection.[7] In most infected birds, virus is detectable first in the spleen, followed by spread to other visceral organs, and then to the CNS.[5] Lesions of WNV in birds are often more extensive in less susceptible species, such as chickens, with multi-organ failure, peracute death and few to no lesions in highly susceptible species like corvids. Chronic, persistent WNV infections have also been documented in some species of birds, including house finches (*Haemorhous mexicanus*) and Western scrub-jays (*Aphelocoma californica*).[7]

In most orders of birds, histologic lesions of WNV are primarily found in the CNS, heart, liver, kidney, and spleen. Typical lesions in the CNS include mononuclear meningo-encephalitis with perivascular cuffing, gliosis, and glial nodules. In other organs, lesions are characterized by lympho-plasmacytic and histiocytic inflammation, accompanied by cellular degeneration or necrosis. While the intestinal lesions seen in the present case, with striking enterocyte and crypt necrosis, have been described in

WNV-infected corvids and other passerines, they have not been described in Anseriformes.[1,2, 4,5, 8] Reports of naturally and experimentally infected geese of various species have emphasized pronounced myocardial lesions and moderate to severe mononuclear meningoencephalitis.[1,2, 4, 8]

JPC Diagnosis: 1. Small intestine: Enteritis, necrotizing, diffuse, severe with crypt abscesses, bar-headed goose, *Anser indicus*. 2. Pancreas: Pancreatitis, necrotizing, random, multifocal, mild.

Conference Comment: This excellent case demonstrates the widespread tissue tropism that West Nile virus (WNV) has in avian species. Most wild birds infected with WNV have a prolonged viremia allowing dissemination of the virus throughout the body, affecting nearly every organ. Typically, microscopic lesions associated with WNV viremia are lymphoplasmacytic and histiocytic inflammation with degeneration and necrosis within the central nervous system (CNS), heart, spleen, kidney, and liver. The virus is distributed throughout the world and outbreaks can cause acute death in a variety of different avian species. Highly susceptible birds include crows, jays, and magpies may die so acutely that there are little to no macroscopic lesions. Chronic disease, characterized by severe inflammatory lesions, emaciation, dehydration, hemorrhage, and congestion, occurs in avian species with lower susceptibility to WNV infection such as owls, hawks, and psittacine birds. Choroiditis, iridocyclitis, and retinal necrosis leading to progressive visual impairment and blindness are reported in naturally WNV infected red-tailed hawks and experimentally infected partridges and pheasants.



Feather follicle bar-headed goose. Necrosis of follicular epithelium was also seen in this individual. (Photo courtesy of: Wildlife Disease Laboratories, San Diego Zoo Institute for Conservation Research, San Diego Zoo Global (WSC 77) http:/institute.sandiegozoo.org/wildlife-disease-laboratories).

As mentioned by the contributor, the virus is transmitted predominantly by *Culex* sp. mosquitoes. Corvid birds, such as the American crow, are the main amplifying host and the virus is maintained in a bird-mosquito-bird lifecycle. Despite being dead end hosts, a variety of mammalian species can be infected, with humans and horses particularly susceptible to developing clinical disease. Transmission occurs during the late spring to early fall during favorable weather conditions for the mosquito vector. In contrast to avian species, lesions in horses are confined to the central nervous system, primarily within the grey matter of the brainstem and thoracolumbar spinal cord and less commonly in the cerebrum and cervical spinal cord. Histologic lesions are typically lymphoplasmacytic meningo-encephalomyelitis with glial nodules, lymphohistiocytic perivascular cuffing, ring hemorrhages, neuronal degeneration, and necrosis. The preferred modality for postmortem diagnosis of WNV infection in mammals includes histologic identification of the previously mentioned lesions, in addition to polymerase chain reaction (PCR) testing of the brainstem for WNV antigens.

Even in severe equine cases, viral antigen is typically scant within the central nervous system making immunohistochemistry (IHC) in-situ hybridization (ISH) less useful in horses.

Ruminants, dogs, cats, and pigs are susceptible to infection, but usually only have transient and subclinical disease; however, a recent report in *Veterinary Pathology* describes severe lympho-plasmacytic meningoencephalitis in six WNV infected sheep with neurological signs in California. In contrast to horses, a large amount of viral antigens accumulated in the CNS of sheep in this study. As a result, both PCR and IHC were useful testing modalities in these sheep. Viral antigens in avian species are widespread and the contributor provides excellent quality images of strong positive cytoplasmic immunoreactivity for WNV antigen within intestinal epithelial cells and crypts and pancreatic acinar cells.

After injection of the virus by the mosquito vector, the virus is deposited in the extracellular matrix where it can propagate in keratinocytes and infect Langerhans dendritic cells and tissue macrophages. The virus then spreads hematogenously via the leukocyte tracking system. Viral envelope proteins E2 and E1 are responsible for organ attachment and endocytosis respectively. Although not completely understood, entry of the virus into the CNS likely involves a combination of breakdown of the blood-brain barrier by proinflammatory cytokines and retrograde axonal transport from the peripheral nervous system.

Contributing Institution:
Wildlife Disease Laboratories
San Diego Zoo Institute for Conservation Research
San Diego Zoo Global
http://institute.sandiegozoo.org/wildlife-disease-laboratories

References:

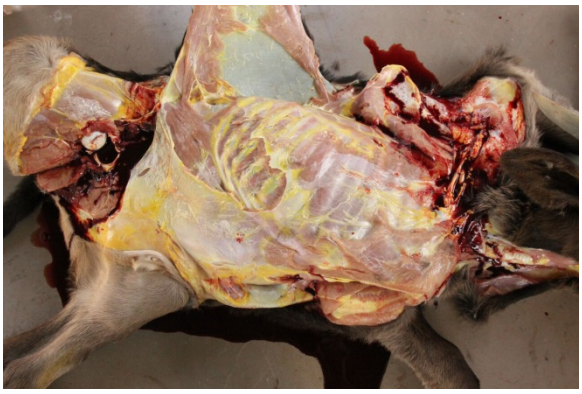
1. Austin RJ, Whiting TL, Anderson RA, Drebot MA. An outbreak of West Nile virus-associated disease in domestic geese (*Anser anser domesticus*) upon initial introduction to a geographic region, with evidence of bird to bird transmission. *Can Vet J*. 2004; 45(2):11-23.
2. Banet-Noach C, Simanov L, Malkinson M. Direct (non-vector) transmission of West Nile virus in geese. *Avian Pathol*. 2003; 32(5):489-94.
1. Cantile C, Youssef S. Nervous system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 1. 6th ed. St. Louis, MO: Elsevier; 2016:374-375.
3. Cox SL, Campbell GD, Nemeth NM. Outbreaks of West Nile virus in captive waterfowl in Ontario, Canada. *Avian Pathol*. 2015;44(2):135-41.
4. Gamino V, Höfle U. Pathology and tissue tropism of natural West Nile virus infection in birds: a review. *Vet Res*. 2013; 44:39.
5. Miller AD, Zachary JF. Nervous System. In: McGavin MD, Zachary JF, eds. *Pathologic Basis of Veterinary Disease*. 6th ed. St. Louis, MO: Elsevier Mosby; 2017:876-877.
6. Nemeth NM, Brown JD, Stallknecht DE, Howerth EW, Newman SH, Swayne DE. Experimental infection of bar-headed geese (*Anser indicus*) and ruddy shelducks (*Tadorna ferruginea*) with a clade 2.3.2 H5N1 highly pathogenic avian influenza virus. *Vet Pathol*. 2013; 50(6):961-70.

7. Pérez-Ramírez E, Llorente F, Jiménez-Clavero MÁ. Experimental infections of wild birds with West Nile virus. *Viruses*. 2014; 6(2):752-81.
8. Swayne DE, Beck JR, Smith CS, Shieh WJ, Zaki SR. Fatal encephalitis and myocarditis in young domestic geese (*Anser anser domesticus*) caused by West Nile virus. *Emerg Infect Dis*. 2001; 7(4):751-3.
9. Zachary JF. Mechanisms of microbial infection. In: Zachary JF ed. *Pathologic Basis of Veterinary Disease.* 6th ed. St. Louis, MO: Mosby Elsevier; 2017:223.
10. Zhou JY, Shen HG, Chen HX, Tong GZ, Liao M, Yang HC, Liu JX. Characterization of a highly pathogenic H5N1 influenza virus derived from bar-headed geese in China. *J Gen Virol*. 2006; 87:1823-33.

CASE III: 15N499 (JPC 4085377).

Signalment: 15-day-old, male, Angus x Nellore cross, ox (*Bos taurus*).

History: On September 2015, in a farm in Midwestern Brazil, eight out of 100, 15-day-old cross bred (Angus x Nelore) calves got sick. Affected calves were unable to follow their mothers and presented with lethargy and ataxia. They were unsuccessfully treated with florfenicol and sodium dipyrone. Five calves died on site; the remaining three sick calves were referred to the Veterinary Teaching Hospital of the Federal University of Mato Grosso do Sul, where they were clinically examined and given supportive therapy. Two of those calves died within 24 hours of the onset of clinical signs and were necropsied. The remaining calf was treated

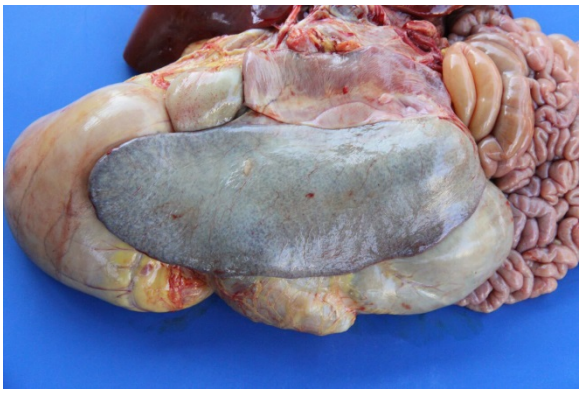


Cadaver, calf. There is moderate subcutaneous icterus. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)

for babesiosis and recovered. Clinical signs in the affected calves included fever, apathy, icterus, stiffness of the neck, and difficulty in keeping a standing position due to incoordination. This latter sign rapidly progressed to sternal decubitus, lateral decubitus, muscle tremors, paddling movements, nystagmus, tachycardia, and tachypnea. One calf had lost of menace reflex and another one had opisthotonus. All calves were parasitized by *Rhipicephalus* (*Boophilus*) *microplus* ticks.

Gross Pathology: In the two necropsied calves (one of each sex), the carcasses were moderated jaundiced, there was splenomegaly and the liver was swollen and had an orange-tan discoloration. The kidneys of one the calves were dark brown and the urine was faint red, although hemoglobinuria was not observed clinically. The grey telencephalic and cerebellar cortices and the basal nuclei had an intense cherry-pink discoloration which contrasted strongly with the white matter. One calf had omphalitis associated with myiasis (*Cochliomyia hominivorax*).

Laboratory results: Blood work: Erythrocytes 4.08 p/10^6µl (reference values 5.5-10.0 p/10^6µl); hemoglobin 6.2 g/dL (8-15 g/dL); mean corpuscular value 50



Spleen, calf. There is moderate splenomegaly. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)

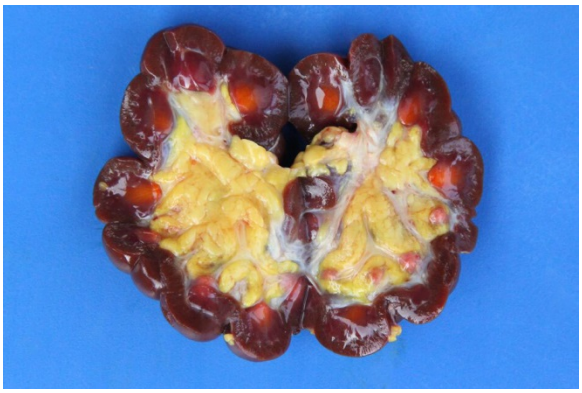
reference values 40-60 fL); Fibrinogen: 1,000 mg/dL (reference values 300-700 mg/dL). Serum biochemistry: Aspartate aminotransferase (AST): 297.5 UI/L (reference values 20 a 34 UI/L); γglutamyl transferase (GGT) 37.4 UI/L (reference values 6.1-7.4 UI/L); glucose 5.8 mg/dL (reference values 45 a 75 mg/dL).
In Romanowsky-stained squashes from telencephalic cortex, capillaries appeared clogged with intraerythrocytic small (2 µm-diameter) paired or single spherical basophilic organisms (morphology compatible with *Babesia bovis*). Similar organisms were also observed within erythrocytes from blood smears.

Histopathologic Description: The most striking lesions are the diffuse and marked capillary engorgement with erythrocytes in the grey matter of the brain. Virtually every erythrocyte within these capillaries contained small paired or single faint basophilic spherical organisms. Perivascular and perineuronal edema are also observed in the grey matter. Mild to minimal mononuclear perivascular cuffings are observed in a few of the slides. There is marked intra-hepatocellular and canalicular cholestasis mainly in centrolobular areas associated with vacuolar hepatocellular degeneration and necrosis. Only minimal hemoglobinuric nephrosis is observed in the kidneys; focal mixed mononuclear cell reaction found in renal interstitium is considered incidental. Changes in the spleen are moderate diffuse congestion. There are no pathological changes in the lungs, heart and gastrointestinal tract.

Contributor's Morphologic Diagnosis: Cerebral cortical congestion, marked, acute, associated with intraerythrocytic organisms with morphology compatible with *Babesia bovis*.

Contributor's Comment: The diagnosis of bovine babesiosis caused by *Babesia bovis* was made based on the pathognomonic gross brain lesions, necropsy and microscopic lesions consistent with the hemolytic crisis, and visualization of the intraerythrocytic parasite in blood imprints, in the capillaries of brain cortical grey matter, in squashes of brain tissue, and HE stained slides.

Babesia was first described by Babés in Romania as a parasite of bovine erythrocytes. Although it is possible for a single *Babesia* species to infect more than one vertebrate host (e.g., *B. microti* affects



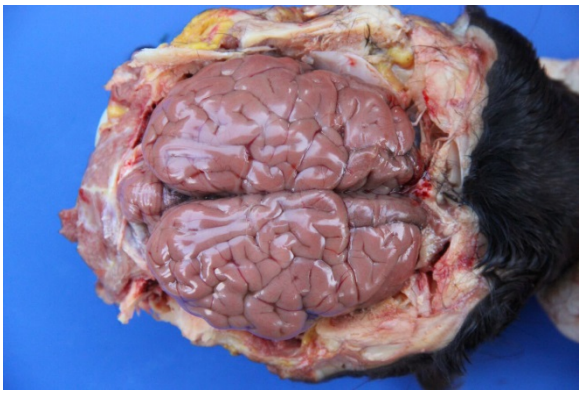
Kidney, calf. The renal cortex is discolored as a result of hemoglobinuria; the pelvis shows profound icterus. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)

rodent and humans; *B. divergens* and *B. bovis* affect cattle and humans), *Babesia* spp. are typically host specific.[2]

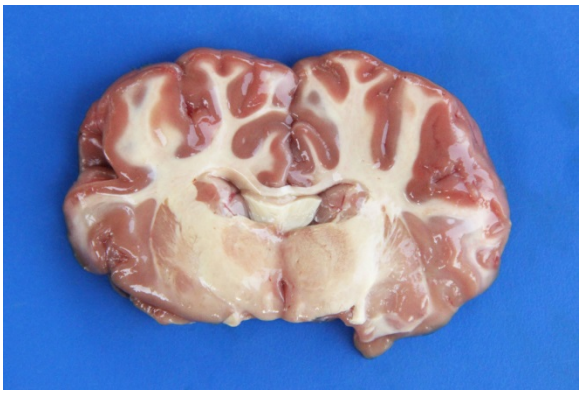
Bovine babesiosis (aka, piroplasmosis, Texas fever, redwater, and tick fever) is a febrile hemolytic condition caused by one of at least seven species the protozoan organisms *Babesia* spp. It is characterized by extensive intravascular hemolysis leading to depression, anemia, icterus, hemoglobinuria, and, in the case of *B. bovis* infections, neurological signs.[2]

In Brazil, bovine babesiosis is caused by *B. bovis* (formerly *B. argentina*) and/or *B. bigemina* and is transmitted to cattle by the tick *Rhipicephalus* (*Boophilus*) *microplus*.[8,10] The disease is frequently, but not always associated with icterus and hemoglobinuria and the animal may become extremely ill before severe anemia, parasitemia or hemoglobinuria are apparent.[5,15]

In general, the disease distribution follows that of the vector ticks producing three distinct epidemiological situations. The disease does not occur in areas without the tick vector. In areas of enzootic instability, there is an alternation of warm and cold seasons. The cold period prolongs the free-living stages of the tick, allowing cattle extended periods without vector contact, resulting in a significant drop in antibodies due to the absence of *Babesia* infection. When the warm period returns, the tick parasite load increases and outbreaks occur. In enzootic areas, weather condition allow the presence of tick on cattle all year round, which confers high level of lasting immunological protection.[1]



Cerebrum, calf. There is a diffuse pink discoloration of the brain as a result of marked cerebral congestion and sequestration of parasitized erythrocytes (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)



Cerebrum, calf. The grey telencephalic cortex and basal nuclei have an intense cherry-pink discoloration which contrast strongly with the subjacent white matter. (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)

Factors influencing the occurrence of babesiosis outbreak include (1) over infestation by vector ticks resulting in a high inoculum of *Babesia*: (2) long periods without ticks with resultant loss of immunity, and (3) stress factor and nutritional deficiencies which can induce a drop in immunity and vulnerability to the disease.[2] Calves are typically more typically more resistant to infection by *Babesia* sp. than adult cattle.[15] Mortality is lower in enzootic areas due to resistance to infection.

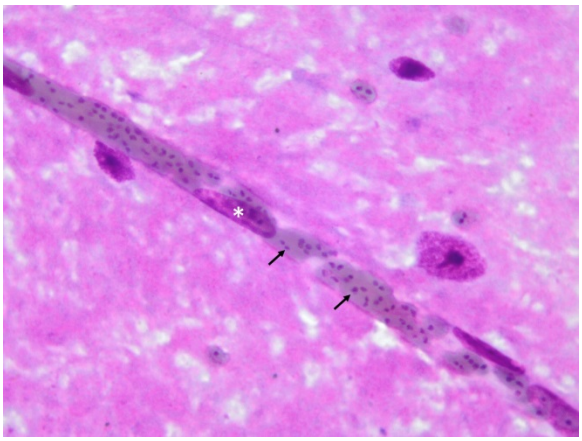
B. bovis is small, pleomorphic apicomplexan parasite, and can occur as single or as paired pear-shaped bodies joined at an obtuse angle within the center of the mature erythrocyte. The round forms measure 1-1.5 µm, and the pear-shaped bodies 1.5 x 2.4 µm in size. Vacuolated signet ring forms are particularly common. Hosts are cattle, buffalo, and deer.[14]

The incubation period for bovine babesiosis is typically 2-3 weeks[1,15] for the natural disease. The natural infection caused by *B. bovis* tends to present a longer incubation time than that caused by *B. bigemina*. Cases of extremely short incubation periods (seven days) have been reported for B. bovis.[13,14]

Infection occurs after the tick vector feeds on the host. After the inoculation of the infectious sporozoites, the parasite penetrates the erythrocytes in the definitive host where they form a parasitophorous vacuole and change to the trophozoite form. Later these trophozoites undergo binary division, usually forming two merozoites. Ticks acquire *Babesia* infection during feeding on infected animals.[2]

Affected cattle develop depression, anorexia, paleness of mucous membranes, and fever (40 degrees/C-42 degrees C). Icterus and hemoglobinuria are also common clinical signs, but they can be minimal or absent in cases of peracute or acute disease. The elevation of serum activity of AST and GGT, observed in this case, may be due to the hepatic centrilobular hypoxic necrosis.[1] Additionally, this calf had omphalitis, which probably resulted in septicemia (high fibrinogen) which would explain the low blood sugar.[4]

B. bovis causes the most severe form of babesiosis in cattle in which peripheral circulatory disturbances with sequestration



Cerebrum, calf. Squash preparation from the telencephalic cortex. An An isolated capillary distended by erythrocytes parasitized by Babesia bovis organisms (arrows). (Photo courtesy of: Laboratory of Anatomic Pathology, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil. https://www.ufms.br/)

of parasitized erythrocytes in the peripheral circulation are unique features.[3,11,12,16] In southern Brazil this form of disease is found in approximately two-thirds of the cases of babesiosis caused by *B. bovis* and is virtually always fatal.[11]

Necropsy findings include yellow discoloration (icterus) of mucosae, subcutaneous tissue, muscle fasciae and the intimal surface of arteries. In acute or peracute cases (which include the cases of cerebral babesiosis) icterus can be mild or absent. The serosal membranes of the abdominal viscera have hemoglobin imbibition. The liver is swollen, with rounded edges, and is yellow or tan discolored. The biliary vesicle is usually markedly distended by dark-green inspissated bile. Subepicardial and subendocardial hemorrhages (petechiae and ecchymosis) are virtually always observed. In cases where hemoglobinuria is a prominent sign, the kidneys are diffusely dark red-brown (hemoglobinuric nephrosis) and the urine is dark-red (red water disease). There is always some degree of splenomegaly. In severely enlarged spleens

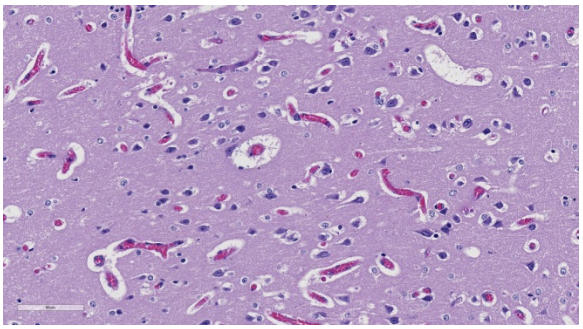
the red pulp prolapses on the cut surface. In cerebral babesiosis, the grey matter of the telencephalic, and cerebellar cortex and that of the basal nuclei has a characteristic cherry pink color. Squashes made from cortical brain, stained with Wright-Giemsa reveal numerous capillaries engorged with parasitized erythrocytes.[3,5,6]

The encephalic lesion is characteristic of *B. bovis* infection and does not occur in any other *Babesia sp*. infection of cattle. However, it can be compared with the brain lesions seen in severe cases of malaria caused by *Plasmodium falciparum*. Microscopically the brain lesions are characterized by cortical capillaries diffusely engorged with red blood cells, and perivascular and perineuronal edema. In tissue sections of the brain, Giemsa and even HE stains demonstrate the parasites as small paired or single spherical faint basophilic bodies.[1,15] Parasitized erythrocytes may also be seen in vessels of virtually all tissues, such as the interstitial capillaries in the kidney, heart, and in skeletal muscle.[15] Other changes characteristic of hemolytic anemia are observed such hemoglobinuric nephrosis, centrilobular hepatic necrosis (due to hypoxia) and bile stasis.[15]

Ultrastructurally, capillaries in the brain are dilated and filled with densely packed parasitized erythrocytes. These erythrocytes have scalloped edges with fine strands apparently connecting adjacent red blood, as well as connecting erythrocytes and endothelial cells.[5,6] Masses of lysed red blood cells which have undergone lysis but still contain intact parasites are frequently seen in capillaries. Changes in the capillary endothelium of the affected parts in the brain range from swelling of the cytoplasm and nucleus to necrosis. Perivascular and perineuronal spaces are enlarged. In the kidneys, capillaries are not packed as tightly with red blood cells as in the brain, and the parasitemia does not exceed 50% of red blood cells. Other changes are similar to those seen in the brain. Capillaries in the lung are packed with red blood cells, but only a small proportion of the cells are parasitized.[5]

Although usually reported resistant to babesiosis, calves in enzootic areas can be parasitized by *R. microplus* on the first day of life.[8] Since the protection of neonates calves is conferred by passive immunity through colostral ingestion, a failure in the transfer of passive immunity from the dam can explain very young calves being affected by the disease. A lack of passive immunity caused by the failure of colostral ingestion in the calf of this report could be suspected since omphalitis was also present.

The pathogenesis of the disease caused by *B. bovis* in cattle is not completely understood, but some mechanisms are proposed.[5,15] Acute disease is characterized by a hypotensive shock syndrome with vascular stasis and accumulation of parasitized erythrocytes in the peripheral circulation. It is accompanied by activation of coagulation/complement cascades and the release of vasoactive compounds resulting in vasodilation and circulatory stasis as well as generalized organ damage due to anoxia and toxic products from both parasites and damaged host tissue.



Cerebellum, calf: Capillaries are diffusely expanded and as a result are more visually prominent than expected within the submitted section. (HE, 224X).

Parasite proteases cause hydrolysis of fibrinogen which results in the accumulation of large quantities of soluble fibrin complexes which are not cross-linked, as well as in altered fibrinogen in the circulation. Thus, the coagulability and viscosity of blood increases but insoluble fibrin is not produced, suggesting that classic disseminated intravascular coagulopathy is not a feature of *B. bovis* infections.[5]

The cause of cytoadherence of in *B. bovis* organisms to endothelial cells is also uncertain,[5,15] but the *Babesia* organisms remodel the erythrocyte surface with their erythrocyte surface antigen proteins, which changes the membrane mechanical and adhesive properties.[15]

In the severe form of malaria caused by *Plasmodium falciparum*, a disease that in many aspects resemble cerebral babesiosis, the causative organism induce infected red cells to clump together and to stick to endothelial cells lining of small blood vessels (sequestration), which blocks blood flow. Several proteins, including *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), associated and form knobs on the surface of erythrocytes. PfEMP1 binds to thrombospondin, VCAM-1, ICAM-1, CD36, and E-selectin on the endothelial cells. Erythrocytes sequestration decreases tissue perfusion and leads to ischemia, which is responsible for the manifestations of cerebral malaria, the major cause of death in children with malaria.[9] Mild to minimal mononuclear perivascular cuffing can be observed in a few of the slides of this case. This change is of no clinical significance encountered in one-third of symptomless cattle.[7]

JPC Diagnosis: Cerebrum, vessesls: Intraerythrocytic protozoal trophozoites, numerous, Angus x Nellore ox, *Bos taurus*.

Conference Comment: The contributor provides an exhaustive review of *Babesia bovis* infection in an ox in addition to excellent quality gross photographs of the striking and pathognomonic uniformly cherry-red cerebral grey matter, referred to colloquially as “cerebral flush” or “pink brain”. This disease is one of the leading causes of infectious mortality of cattle in Brazil.[2,15] As mentioned by the contributor, this pink discoloration of the brain is secondary to marked congestion of the cerebral microvasculature by infected erythrocytes. In this case, nearly every erythrocyte is infected with at least one round to pyriform trophozoite. Parasitized erythrocytes have a tropism for the cerebral grey matter, kidney, heart, and skeletal muscle, but infected erythrocytes can be found in any tissue. Interestingly, the parasite is only rarely found in about 5% of circulating red blood cells.[15]

The sequestration of infected erythrocytes within the microvasculature of the cerebrum and other visceral organs leads to occlusion of the vessels and subsequent hypoxic injury. *Babesia bovis* also releases vasoactive proteases that activate the hypotensive agent, kallikrein, which then activates another potent vasodilator, bradykinin.[15] The dilatory effect of these

vasoactive proteins shifts blood away from veins and further contributes to apparent anemia. Additionally, infected animals are typically markedly anemic secondary to both intravascular and extravascular hemolysis, described by the contributor.[15] The combination of vascular congestion, vasodilation, and hemolysis leads to both metabolic alkalosis and a hemodynamic crisis, often resulting in the death of the animal.

Most conference participants noted congestion and dilation of the cerebral microvasculature in this case; however, some attendees offered a dissenting opinion that the apparent congestion is a result of post-mortem pooling of blood within the brain rather than an antemortem change. The conference moderator agreed with the majority of the participants that the cerebral congestion is part of the pathogenesis of this disease and likely represents a real lesion, rather than an artifact. Participants also astutely noted that while vessels are congested in both the grey and white matter histologically, congestion is only apparent within the grey matter macroscopically.[15]

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References:

1. Barros CSL, Driemeier D, Dutra IS, Lemos RAA. Babesiose cerebral [Cerebral babesiosis]. In: Barros CSL, Driemeier D, Dutra IS, Lemos RAA, eds. *Doenças do sistema nervoso de bovinos no Brasil* [Diseases of the central nervous system in cattle in tick Brazil]. São Paulo, SP: Coleção Vallée; 2006:87-95.
2. Barros CSL, Fighera RA. Babesiosis. In: *Foreign animal diseases*. Washington DC, United States: Animal Health Association, 2008:147-157.
3. Callow LL, McGavin MD. Cerebral babesiosis due to *Babesia argentina*. *Aust Vet J*. 1963; 39:15-21.
4. Carlson GP. Clinical chemistry tests. In: Smith BP, ed. *Large Animal Internal Medicine*. 4th ed. St. Louis: Mosby; 2009:393.
5. de Vos AJ, de Waal DT, Jackson LA. Bovine babesiosis. In: Coetzer JAW, Tustin RC, eds. *Infectious Diseases of Livestock*. 2nd ed., vol. 1. Cape Town: Oxford University; 2004:406-424.
6. Everitt JI, Shadduck JA, Steinkamp C, Claubaugh G. Experimental *Babesia bovis* infection in Holstein calves. *Vet Pathol*. 1986;23:556-562.
7. Gavier-Widen D, Wells GAH, Simmons MM, Wilesmith JWW, Ryan J. Histological observations on the brains of symptomless 7-year-old cattle. *J Comp Path*. 2001; 124:52-59.
8. Kessler RH, Madruga CR, Schenk MAM Ribeiro OC. Babesiose cerebral por *Babesia bovis* em bezerros no Estado de Mato Grosso do Sul [Cerebral babesiose by *Babesia bovis* in calves in Mato Grosso do Sul]. *Pesq Agropec Bras*. 1983;18:931-935.
9. McAdams AJ, Milner, Sharp AH. Parasitic infections. In: Kumar V, Abbas A, Aster J, eds. *Robbins & Cotran Pathologic Basis of Disease*. 9th ed. Philadelphia: Elsevier Saunders; 2014:389-402.
10. Patarroyo JH, Vargas M.L., Bicudo PL. Description of lesions in cattle in

a natural outbreak of *Babesia bovis* infection in Brazil. *Vet Parasitol*. 1982;11:301-308.
11. Rodrigues A, Rech RR, Barros RR, Fighera RA, Barros CSL. Babesiose cerebral em bovinos: 20 casos [Cerebral babesiosis in cattle: 20 cases]. *Ciência Rural*. 2005;35:121-125.
12. Rogers RJ. Observations on the pathology of *Babesia argentina* infections in cattle. *Aust Vet J*. 1971; 47:242-247.
13. Santarosa BP, Dantas GTN, Ferreira DOL, et al. Infecção neurológica por *Babesia bovis* em bovino neonato – relato de caso [*Babesia bovis* neurological infection in bovine neonates - case report]. *Vet. Zootec*. 2013; 20:9-14.
14. Taylor MA, Coop RL, Wall RL. *Veterinary parasitology*. 3rd ed. Oxford: Blackwell; 2007:105-107.
15. Valli VEO, Kiupel M, Bienzle D. Babesiosis. In: Maxie MD, ed. *Jubb, Kennedy & Palmer Pathology of Domestic Animals*. 6th ed. vol. 3. St. Louis, MO: Elsevier; 2016:118-120.
16. Zlotnic L. Cerebral piroplasmosis in cattle. *Vet Rec*. 1953; 40:642-643.

CASE IV: 14-9 (JPC 4087119).

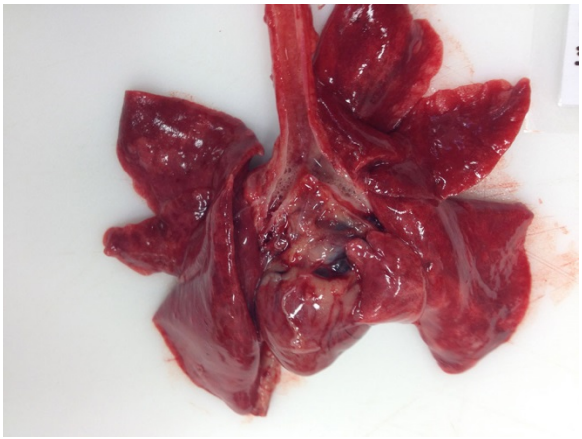
Signalment: Five-year-old female ragdoll mix cat (*Felis catus*).

History: The cat was found dead at home in January 2014, after one day of anorexia, open-mouth breathing, and hiding. The cat had lived with two littermates and had no outdoor access. On the day before this cat's death, a littermate from the same home had developed severe respiratory distress and been euthanized but not necropsied. The third littermate never became ill and is still alive 2.5 years later at the time of submission.

Gross Pathology: At necropsy, both lungs were diffusely consolidated and edematous. Formalin-fixed slabs of lung had prominent, grossly visible, pale cuffs around bronchioles. There were no other gross abnormalities in any organ system.

Laboratory results: Samples of lung were submitted to a commercial diagnostic laboratory for a feline upper respiratory disease panel. This panel was negative by real-time PCR for all agents tested: feline calicivirus, *Chlamydophila felis*, feline herpesvirus 1, *Bordetella bronchiseptica, Mycoplasma felis* and H1N1 influenza virus.

Histopathologic Description: Lung: In most bronchioles, the lining epithelium is segmentally to completely sloughed or attenuated, with occasional attempts at regeneration. The lumens of affected bronchioles frequently contain adherent strands of fibrin and cellular debris



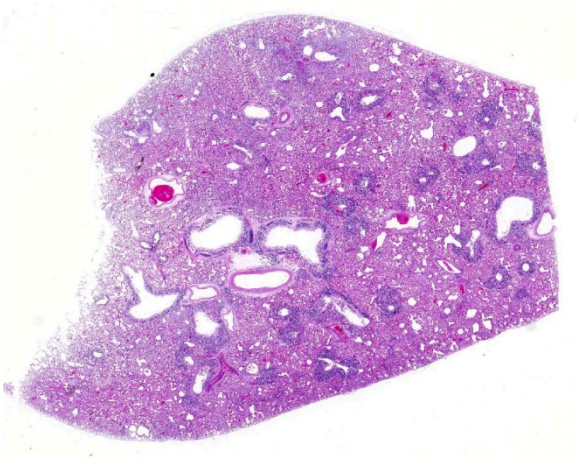
Lungs, cat. All lung fields are consolidated and edematous. (Photo courtesy of: Department of Veterinary Clinical and Diagnostic Sciences, Faculty of Veterinary Medicine, University of Calgary, 3280 Hospital Dr. NW, Calgary, Alberta T2N 4Z6, Canada, http://vet.ucalgary.ca/)

composed of macrophages, sloughed epithelial cells, and free red blood cells.

Diffusely, alveolar airspaces contain moderate numbers of cells, predominantly macrophages and lymphocytes, with fewer non-degenerate neutrophils and red blood cells. In addition, alveolar airspaces contain abundant eosinophilic beaded to fibrillar material (fibrin). Numerous peribronchiolar alveoli are lined by markedly hypertrophied cuboidal epithelial cells with large nuclei and prominent nucleoli (type II pneumocyte hyperplasia). Multinucleate pneumocytes are occasionally seen.

Around larger pulmonary vessels, connective tissue is moderately expanded by clear space (perivascular edema). Throughout all sections, muscular pulmonary arteries and arterioles have moderately thickened walls (smooth muscle hyperplasia).

Contributor's Morphologic Diagnosis: Lung: Severe, diffuse, acute to subacute, multifocal to coalescing, necrotizing bronchointerstitial pneumonia with alveolar edema and marked peribronchiolar type II pneumocyte hyperplasia.



Lung, cat. There is marked hypercellularity surrounding airways. (HE, 5X)

Contributor's Comment: This cat died from severe bronchointerstitial pneumonia, which is most frequently caused by feline herpesvirus or feline calicivirus infection.[4] Lung samples were submitted to a commercial diagnostic laboratory and no viral or bacterial sequences were detected using real time PCR. However, because this cat's gross and histologic lesions were nearly identical to those described previously in two cats that died of influenza A(H1N1)pdm09 virus infection (so-called pandemic influenza),[8] we submitted samples to a provincial diagnostic laboratory, which detected this virus. This is, to our knowledge, the first confirmed case of influenza A(H1N1)pdm09 infection in a Canadian cat.

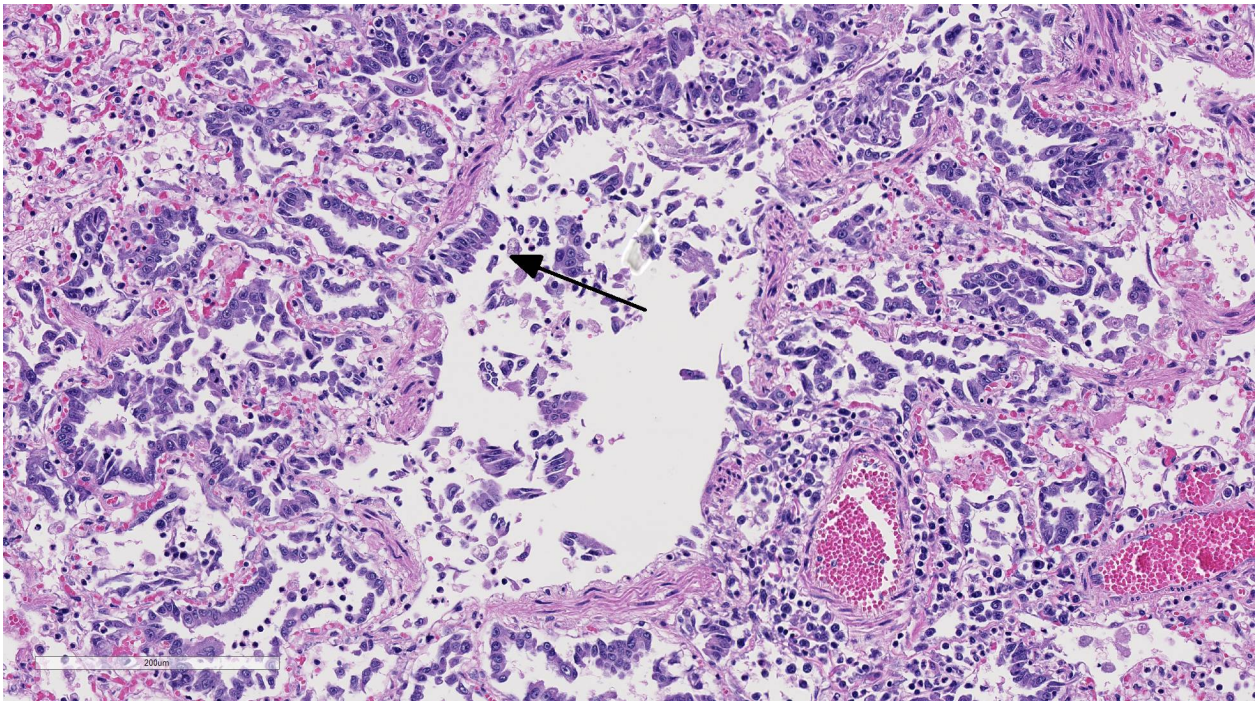
Influenza A viruses are RNA viruses in the family *Orthomyxoviridae* that can infect multiple species of mammals and birds, although different viral subtypes tend to be host-specific.[17] These viruses have caused multiple epidemics and pandemics in human populations,[11] as well as epizootics and panzootics in animal species.[12,16] Rapid mutation and gene reassortment of influenza A viruses lead to a high diversity of viral subtypes, and this genetic flexibility results in a propensity for between-species and cross-class transmission.[18] The so-called pandemic strain of the H1N1 influenza virus isolated in 2009 (influenza A(H1N1)pdm09) contained a novel combination of genetic segments from influenza viruses affecting humans, pigs, and birds.[6]

Cats are susceptible to infection by multiple influenza A virus subtypes;[1] however, reports of clinical disease in cats resulting from natural infection with A(H1N1)pdm09 are relatively scarce.[3,5,8,10,13] The source of this particular cat's infection could not be determined. The owner lived alone, had received a seasonal influenza vaccination in

October of 2013, and had never become sick. However, she had hosted two house guests for a three day period after Christmas 2013, approximately 10 days before the deaths of her two cats in early January, 2014. One guest had "flu-like" symptoms and remained indoors for the majority of the visit. Although the cause of this guest's illness was never known, the 2013–2014 influenza season was intensive in Alberta; there were 35% more lab-confirmed cases of human influenza infection than the previous year and the predominant circulating strain was influenza A(H1N1)pdm09.[14] Thus, the sick guest could not be ruled out as the source of this cat's, and/or her littermate's, infection.

The possibility of anthroponotic transmission of influenza A(H1N1)pdm09 to cats is supported by two earlier reports. The first describes an indoor-only cat that was infected with influenza A(H1N1)pdm09 after exposure to human family members with a non-diagnosed flu-like illness.[13] The second shows that pet cats are nearly three times more likely to be seropositive for influenza A(H1N1)pdm09 than free-roaming cats.[18] Cat-to-cat transmission of influenza A(H1N1)pdm09 has also been documented, both experimentally[15] and in an outbreak in cats with little human contact living in a cat colony in Italy.[5] Zoonotic transmission of influenza viruses from cats to humans may also occur, and the role that cats may play in the epidemiology of human influenza infections needs further investigation.[1]

The cat described here died from severe pneumonia caused by pandemic influenza A virus infection. This virus was not detected in samples sent to a commercial laboratory, but was detected in samples sent to a second laboratory. Therefore, if clinical signs, history (exposure to people or animals

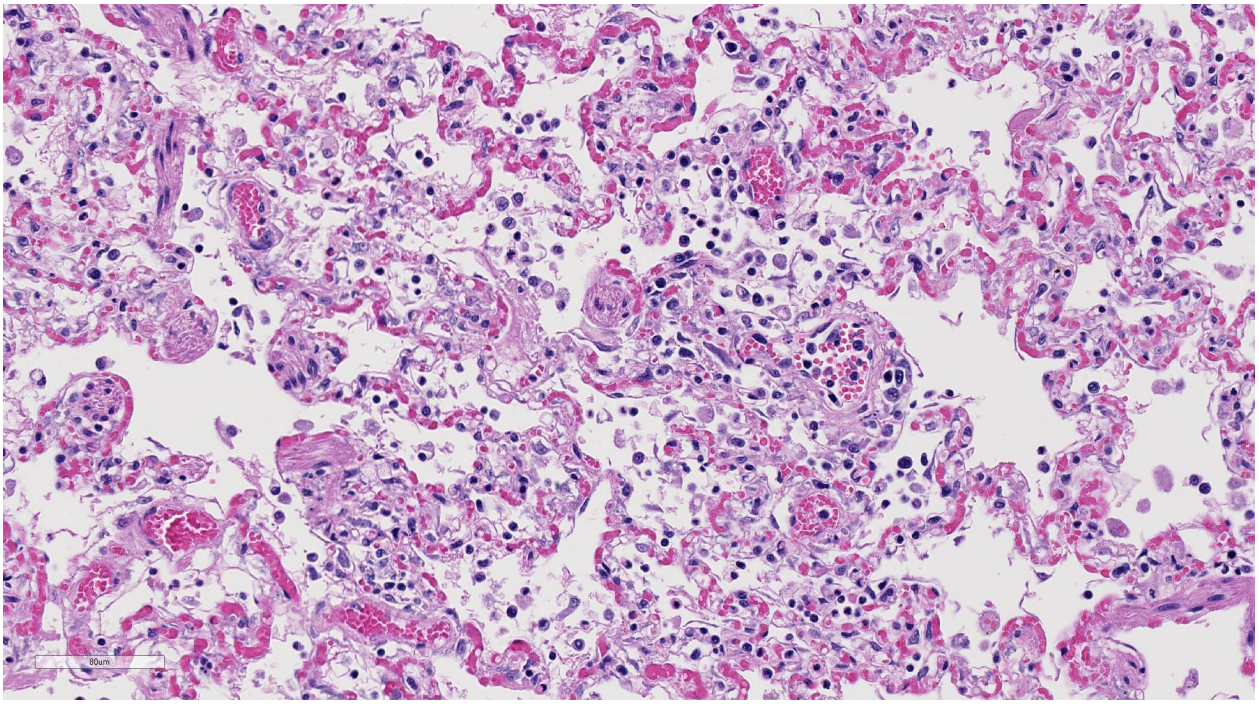


Lung, cat. Diffusely, airway epithelium is necrotic and almost totally sloughed. There is marked bronchiologenic type II pneumocyte hyperplasia. (HE, 104X)

infected with influenza virus), gross lesions, and histologic findings are suggestive of influenza virus infection, it is reasonable to test samples in a second laboratory should a first laboratory provide a negative result for influenza virus infection. This case highlights the importance of including influenza virus infection in the differential diagnosis for respiratory disease in cats. It also further demonstrates the importance of investigating any discrepancy between histologic findings and expected laboratory results.

JPC Diagnosis: Lung: Pneumonia, bronchointerstitial, necrotizing, multifocal to coalescing, severe with marked peri-bronchiolar type II pneumocyte hyperplasia, ragdoll mix cat, *Felis catus*.

Conference Comment: The diagnosis of bronchointerstitial pneumonia was the subject of much debate among conference participants. It is usually reserved for cases where there is microscopic evidence of viral targeting of both the bronchiolar and alveolar epithelium, which is the typical behavior of aerogenous viral infections or inhaled toxins.[4] Damage to the airway epithelium and alveolar septa causes epithelial necrosis and hyperplasia of type II pneumoncytes.[4] This case is an excellent example of bronchointerstitial pneumonia with profound multifocal bronchiolar-centric type II pneumocyte hyperplasia, nicely highlighted by pancytokeratin immuno-histochemical stain run by the Joint Pathology Center prior to the conference. Interestingly, marked type II pneumocyte hyperplasia can occur as early as two days after initial insult to the respiratory epithelium and alveolar septum, coinciding with the acute onset reported in the history of this case.[7,8] Conference participants discussed other differential diagnoses for bronchointerstitial pneumonia in cats, such



Lung, cat. Diffusely, alveoli are expanded by moderate amounts of fibrin, congestion, and edema. Alveolar spaces contain variable combinations and concentrations of inflammatory cells, polymerized fibrin and cellular debris. (HE, 228X)

as feline herpesvirus-1 and feline calicivirus.[4,7,8]

The conference moderator also noted that in this case the virus appears to exhibit a tropism for terminal airways with relative sparing of the larger bronchi. The specific anatomic location of lesions associated with influenza virus has been reported to correlate with the distribution of sialic acid molecules on the cell surface of the host.[8,9] In human pandemic H1N1 influenza A, these sialic acids are distributed in the trachea and larger airways, resulting in necrotizing tracheitis and bronchitis in severely affected human patients. In pathogenic avian influenza H5N1, the virus binds to sialic acids present mainly on terminal bronchioles and pneumocytes.[8,9] Interestingly, the distribution of lesions in this case differs from the typical presentation of human H1N1 influenza A, and resembles binding of avian influenza viral particles. This distribution pattern has been reported in other cases of H1N1 infection in cats.[8]

Influenza viruses are RNA viruses of the *Orthomyxovridiae* family, and are able to rapidly mutate through antigenic drift and genetic reassortment.[1,7,8,9] This leads to a high degree of viral diversity and genetic flexibility, thus allowing the virus to quickly adapt and infect multiple different species.[7,8] In addition to human origin H1N1, presented in this case, cats are also susceptible to highly pathogenic avian influenza H5N1 and H7N2 viruses.[4] Cats have been reported to be infected with H1N1 by interaction with infected humans, as is suspected in this case, and then transmit the disease horizontally to other felids. There are currently no reports of transmission from cat to human with H1N1 virus.[7,8] Infection with highly pathogenic avian influenza in cats is thought to be secondary to both inhalation of aerosolized virus and consumption of infected birds.[4]

In December 2016, there was an outbreak of H7N2 avian influenza in almost 400 shelter cats in New York City.[2] A shelter veterinarian who had prolonged and unprotected exposure to affected cats was also infected with the virus. This is the first documented case of cat to human transmission of influenza and only the third case of human H7N2 avian influenza infection in the United States.[2] Given their close relationship with humans and susceptibility to both human and avian influenza strains, cats have the potential to be important reservoirs for infection.[2,7,8] Feline co-infection with human and avian strains may allow genetic reassortment and antigenic shift creating a novel influenza A subtype and provide the basis for a new influenza pandemic.[7] As a result, influenza virus should be carefully considered as a differential diagnosis for respiratory disease in cats.

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References:

1 Ali A, Daniels JB, Zhang Y, Rodriguez-Palacios A, Hayes-Ozello K, Mathes L, Lee C-W. Pandemic and seasonal human influenza virus infections in domestic cats: Prevalence, association with respiratory disease, and seasonality patterns. *J Clin Microbiol*. 2001; 49:4101-4105.

2 Avian influenza A (H7N2) in cats in animal shelters in NY; one human

infection. Centers for Disease Control and Prevention website. https://www.cdc.gov/flu/spotlights/avian-influenza-cats.htm. Updated January 4, 2017. Accessed February 16, 2017.

3 Campagnolo ER, Rankin JT, Daverio SA, Hunt EA, Lute JR, Tewari D, Acland HM, Ostrowski SR, Moll ME, Urdaneta VV, Ostroff SM. Fatal pandemic (H1N1) 2009 influenza A virus infection in a Pennsylvania domestic cat. *Zoonoses Public Health*. 2011; 58:500-507.

4 Caswell JL, Williams KJ. Respiratory system. In: Maxie MG ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol 2. 6th ed. St. Louis, MO: Elsevier; 2016:511,587-591.

5 Fiorentini L, Taddei R, Moreno A, Gelmetti D, Barbieri I, De Marco MA, Tosi G, Cordioli P, Massi P: Influenza A pandemic (H1N1) 2009 virus outbreak in a cat colony in Italy. *Zoonoses Public Health*. 2011; 58:573-581.

6 Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu XY, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivailler P, Smagala J, de Graaf M, Burke DF, Fouchier RAM, Pappas C, Alpuche-Aranda CM, Lopez-Gatell H, Olivera H, Lopez I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM, Dotson PD, Boxrud D, Sambol AR, Abid SH, George KS, Bannerman T, Moore AL, Stringer DJ, Blevins P, Demmler-Harrison GJ, Ginsberg M, Kriner P, Waterman S, Smole S, Guevara HF, Belongia EA, Clark PA, Beatrice ST, Donis R, Katz J, Finelli L, Bridges CB, Shaw M, Jernigan DB, Uyeki TM, Smith DJ, Klimov AI, Cox NJ. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science*. 2009; 325:197-201.

7 Knight CG, Davies JL, Joseph T, Ondrich S, Rosa BV. Pandemic H1N1 influenza virus infection in a Canadian cat. *Can Vet J*. 2016; 57(5):497-500.

8 Lohr CV, DeBess EE, Baker RJ, Hiett SL, Hoffman KA, Murdoch VJ, Fischer KA, Mulrooney DM, Selman RL, Hammill-Black WM. Pathology and viral antigen distribution of lethal pneumonia in domestic cats due to pandemic (H1N1) 2009 influenza A virus. *Vet Pathol*. 2010; 47:378-386.

9 Marschall J, Hartmann K. Avian influenza A H5N1 infections in cats. *J Fel Med Surg*. 2008; 10:359-365.

10 Pigott AM, Haak CE, Breshears MA, Linklater AKJ. Acute bronchointerstitial pneumonia in two indoor cats exposed to the H1N1 influenza virus. *J Vet Emerg Crit Care*. 2014; 24:715-723.

11 Pleschka S. Overview of influenza viruses. In: Richt JA, ed. *Current Topics in Microbiology and Immunology: Swine Influenza*. Webby RJ: Springer-Verlag, Berlin; 2013:1-20.

12 Schultz-Cherry S, Olsen CW, Easterday BC. History of swine influenza. In: Richt JA, ed. *Current Topics in Microbiology and Immunology: Swine Influenza*. Webby RJ: Springer-Verlag, Berlin; 2013:21-28

13 Sponseller BA, Strait E, Jergens A, Trujillo J, Harmon K, Koster L, Jenkins-Moore M, Killian M, Swenson S, Bender H, Waller K,

Miles K, Pearce T, Yoon KJ, Nara P. Influenza A pandemic (H1N1) 2009 virus infection in domestic cat. *Emerg Infect Dis*. 2010; 16:534-537.
14 Surveillance and Assessment Branch AH: Seasonal Influenza in Alberta – 2012/2013 Summary Report. http://www.health.alberta.ca/documents/Influenza-Summary-Report-2014.pdf. Edmonton, Alberta, 2014.
15 van den Brand JMA, Stittelaar KJ, van Amerongen G, van de Bildt MWG, Leijten LME, Kuiken T, Osterhaus A. Experimental Pandemic (H1N1) 2009 virus infection of cats. *Emerg Infect Dis*. 2010; 16:1745-1747.
16 Vijaykrishna D, Bahl J, Riley S, Duan L, Zhang JX, Chen HL, Peiris JSM, Smith GJD, Guan Y. Evolutionary dynamics and emergence of panzootic H5N1 influenza viruses. *PloS Path*. 2008; 4:10.
17 Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev*. 1992; 56:152-179.
18 Zhao FR, Liu CG, Yin X, Zhou DH, Wei P, Chang HY. Serological report of pandemic (H1N1) 2009 infection among cats in Northeastern China in 2012-02 and 2013-03. *Virol J*. 2014; 11:4.