The Armed Forces Institute of Pathology
Department of Veterinary Pathology

Conference Coordinator:
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WEDNESDAY SLIDE CONFERENCE 2008-2009

Conference 8

5 November 2008

Conference Moderator:

Dr. Matthew Starost, DVM, PhD, Diplomate ACVP

CASE I – A8-043171 (AFIP 3106959)

Signalment: 15-month-old, female, brangus (*Bos taurus x Bos indicus*)

History: The animal was purchased one week prior to necropsy in Texas and transported to Georgia in April 2008. In Texas, it had been in contact with exotic ruminants, including wildebeest and sheep. Initial clinical signs included keratitis, nasal discharge, and fever. When hospitalized, additional signs included cranial nerve deficits, rumen hypomotility, and generalized lymphadenopathy. A head tilt developed during the course of treatment and euthanasia was elected.

Gross Pathology: The carcass was in good physical condition and the uterus contained a normal, approximately 4-5 month old fetus. Eyelids contained multifocal to coalescing hyperemic foci and there was bilateral corneal opacity and turbid exudates in the anterior chambers. The nares had bilateral viscous white discharge. Turbinates were severely congested and covered by moderate amounts of fibrin. Multiple erosions on the tongue had underlying hyperemia. Mandibular, prescapular, left axillary, tracheobronchial, and mediastinal lymph nodes were severely enlarged. Parenchyma bulged from the cut surface and was hemorrhagic or had multiple small hyperplastic white nodules in the cortex and medulla. Lungs were diffusely wet and edematous and the trachea contained abundant white froth. The liver was diffusely mottled and margins were rounded.

Laboratory Results:
FA negative for rabies virus and *Listeria*
IFA positive for malignant catarrhal fever virus (lymph node)
PCR positive for AlHV-1 and OHV-2 malignant catarrhal fever viruses

Histopathologic Description: Arterial walls of the rete mirabilis are infiltrated by moderate to large numbers of lymphoid cells that accumulate predominantly at the outer margins of the tunica media and in the adventitia **(Fig. 1-1)**. Inflammatory infiltrates are predominated by lymphocytes with moderate amounts of finely granular pale eosinophilic cytoplasm and large nuclei, with marginated chromatin and prominent nucleoli **(Fig. 1-2).** Similar infiltrations are present less frequently in the subendothelial connective tissue and rarely are present transmurally. Although the severity of changes is variable between individual sections, the media of multiple arteries is expanded by amorphous eosinophilic material (fibrinoid necrosis) and foci of pyknotic debris.

Contributor's Morphologic Diagnosis: Severe, multifocal, lymphocytic and lymphoblastic vasculitis and

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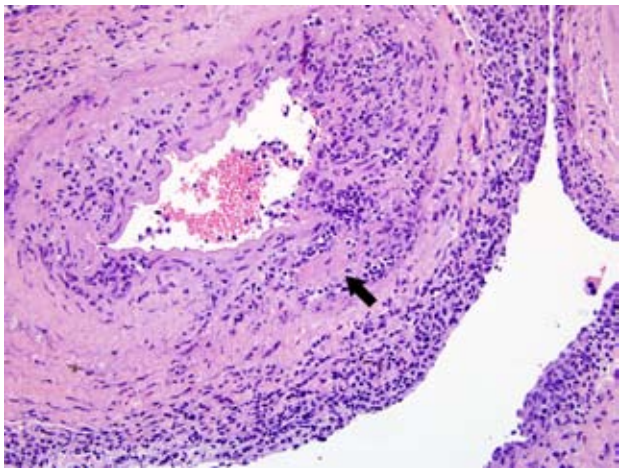
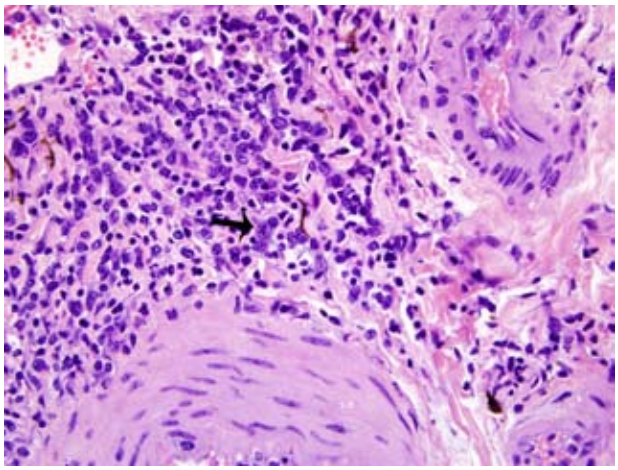


1-1. Ox, rete mirabilis. Diffusely there is variably dense periarteriolar lymphoid proliferation with cellular infiltrate extending into and segmentally disrupting the tunica adventia, media, and intima. Focally, there is an area of fibrinoid necrosis of the tunica media characterized by brightly eosinophilic fibrillar material surrounded by moderate numbers of lymphocytes, plasma cell and histiocytes (arrow). (HE 200X).



1-2. Ox, rete mirabilis. Within periarteriolar lymphoid aggregates there are moderate numbers of lymphoblasts characterized by large vesiculate nuclei containing a prominent 5-6 micron eosinophilic nucleolus (arrow). (HE 400X).

perivasculitis with fibrinoid necrosis, rete mirabilis

Contributor's Comment: Gross and microscopic findings were consistent with malignant catarrhal fever (MCF). The predominant lesion in this heifer, severe lymphocytic and necrotizing vasculitis, was present in multiple tissues including the heart, uterus, forestomachs, abomasums, tongue, kidney, and liver. Lesions were also present in the brain and were particularly evident in the submitted rete mirabilis surrounding the pituitary. Fluorescent antibody testing performed on lymph node was positive, but does not differentiate between MCF associated viruses. PCR testing performed at the NVSL in Ames, Iowa yielded sequences compatible with both the alcelaphine and ovine forms of the MCF virus.

MCF is a pansystemic lymphoproliferative disease syndrome of certain wild and domestic artiodactylid species, occurs worldwide, and is caused by several closely related members of the *Rhadinovirus* genus of gamma-herpes viruses that exist in nature in subclinical infections in carrier ruminants.[7] Although no clinicopathologic differences exist between the diseases, four viruses are associated with MCF: 1) Alcelaphine herpesvirus 1 (AlHV-1) carried by wildebeest; 2) Ovine herpesvirus 2 (OHV-2) endemic in domestic sheep; 3) Caprine herpesvirus 2 (CpHv-2) endemic in domestic domestic goats; and 4) an undetermined virus in white-tailed deer (MCFV-WTD).(3,4) Unlike the alpha- and betaherpesvirus, the gammaherpesvirus appear to favor the establishment of latency over lytic replication in most infected cells of their natural hosts. In return for protecting their latently infected cells from immune system destruction, these reservoir hosts have evolved to being subclinical transmitters of the virus. However, a balance must exist between the immune response and the number of infected cells. Virus excretion is usually low and may occur constantly or intermittently. Animals targeted by the virus that did not co-evolve with it, as well as natural hosts with immune deficiencies, lose control over numbers of latently infected cells and develop lethal disease.[1]

First isolated and identified in 1960 from wildebeest, AlHV-1 is restricted to Africa and zoological collections where wildebeest are present.[5] OHV-2 occurs worldwide, but has never been isolated in cell culture. Natural infections can occur in goats, cattle, bison, deer, and pigs.[1,2] Clinical signs and prominent gross changes include lymphadenopathy, eyelid edema and conjunctivitis, corneal opacity and ulceration, hypopyon, oculonasal discharge, congestion of nasal mucosa, oral and esophageal erosion to ulceration, exudative dermatitis, sloughing of horns and hooves, and nervous signs. The primary microscopic lesions in ruminants with acute MCF are lymphoid proliferation and infiltration, necrotizing

vasculitis with perivascular lymphoid infiltration, and necrosis of mucosal epithelium.[3,7] In situ, PCR and immunohistochemistry studies with OHV-2 have demonstrated that the predominating infiltrating cell type is the CD8+ T-lymphocyte and that large numbers are infected. Cases of MCF are generally sporadic and the disease is not contagious among cattle by direct contact, making them dead end hosts. The incubation period is usually 2-10 weeks, but may be much longer.[3]

AFIP Diagnosis: Rete mirabilis: Arteritis and periarteritis, necrotizing, lymphocytic, multifocal, marked with fibrinoid necrosis

Conference Comment: Malignant catarrhal fever (MCF), also referred to as snotsiekte or malignant head catarrh, is an important disease in ungulates and should be included on a differential list of ulcerative, mucosal diseases in ruminants. Other differentials include: rinderpest (bovine morbillivirus), bovine viral diarrhea (bovine pestivirus), foot and mouth disease (bovine aphthovirus), bluetongue (orbivirus), bovine papular stomatitis virus (bovine parapox) and infectious bovine rhinotracheitis (bovine herpesvirus-1).

The most characteristic features of MCF are proliferation of CD8+ T-lymphocytes, vasculitis, and respiratory and gastrointestinal ulceration. The virus infects large granular lymphocytes which have T-suppressor cell and natural killer cell activity. Viral infection of these cells is thought to cause lymphoproliferation (due to suppressor dysfunction) and necrosis (due to natural killer cell dysfunction).[3] Disease progression can be as short as 1-3 days. This short clinical progression usually manifests as a hemorrhagic enteritis. Animals with less severe gastrointestinal disease often have neurologic disease combined with general debilitation and die within 10 days of disease onset.[3]

Grossly, MCF is characterized by mucosal ulceration in the upper portions of the respiratory tract, which coincides with the copious nasal discharge sometimes seen clinically. Skin lesions are often seen in sheep-associated MCF but are often overlooked during the post-mortem. Like rinderpest, esophageal erosions and ulcers are more common in the proximal third of the gastrointestinal tract. Ocular lesions include edema of the eyelids and conjunctiva and corneal opacity.[3]

The kidney may be mottled secondary to infarction or interstitial nonsuppurative nephritis. The urinary bladder can also be affected with lesions similar to those seen in the kidney, or the mucosa may be ulcerated causing hemorrhage and hematuria. As mentioned previously, lymph node as well as hemal node enlargement is prominent as a result of lymphoid hyperplasia. The spleen also contains prominent lymphoid follicles. Neurologic disease is common in subacute and chronic cases and is secondary to vasculitis.[3]

Contributing Institution: Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, http://www.vet.uga.edu/VPP/index.php

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CASE II – 06-17642 (AFIP 3106254)

Signalment: A 1-year-old female domestic long hair (DLH) cat (*Felis domesticus*)

History: The cat was 5-6 weeks pregnant and died after partial abortion (aborting three of six fetuses). Fixed tissues in 10% buffered formalin and blood smears were received at Tifton Veterinary Diagnostic and Investigational Laboratory.

Gross Pathology: NA

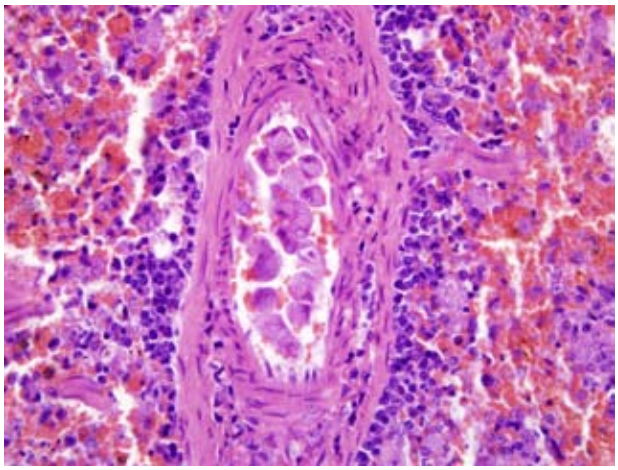
Laboratory Results: NA

Histopathologic Description: Within sections of kidney, liver and spleen, veins, arterioles and arteries contained few to several foamy macrophages which occasionally partly occluded the vascular lumens **(Fig. 2-1)**. The macrophages are enlarged up to twice normal size and contained cytoplasmic schizonts with numerous 1-2 micron in diameter round to oval bluish organisms (merozoites). Similar macrophages containing protozoan schizonts were seen within several glomerular capillaries **(Fig. 2-2)**. The schizont-laden macrophages were seen attached to endothelium, at times occluding blood vessels, and were numerous especially in sections from the spleen and liver **(Figs. 2-3, 2-4)**.

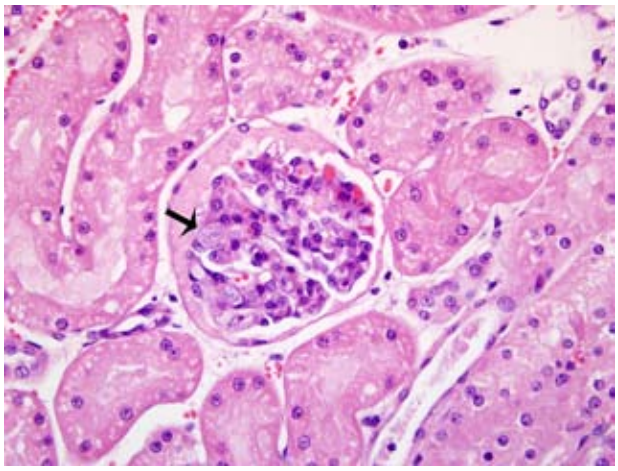
Additionally, tissues (slides not submitted) from heart, lung, stomach, small intestine, ovary and urinary bladder and gall bladder were examined. In almost all examined tissues, there were similar large schizont-laden macrophages in the blood vessels. These were numerous in the sections of liver, kidney, spleen and heart. The other findings were within normal limits.

Contributor's Morphologic Diagnosis: Kidney, spleen and liver, blood vessels (veins, arteries and arterioles): Histiocytosis, diffuse, moderate with intrahistiocytic intracytoplasmic schizonts, morphology most consistent with *Cytauxzoon felis* (a Theilerial parasite)

Contributor's Comment: *Cytauxzoon felis* is a protozoan parasite classified in family *Theileriidae* and infects wild ungulates species in Africa including the kudu, eland and giraffe, as well as domestic and wild *Felidae* in North America.[3] The organism is believed to be transmitted from bobcat, the reservoir host, to domestic cats via a tick vector.[1] The clinical disease in domestic cat is most prevalent in the early and late summer, corresponding with the activity of its tick vector,



2-1. Cat, spleen. Filling splenic vessels are numerous monocytes containing high numbers of Cytauxzoon felis schizonts. (HE 200X).

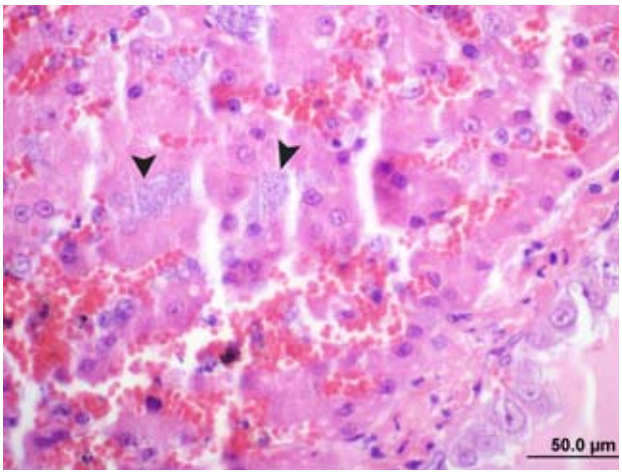


2-2. Cat, kidney. Multifocally, within the glomerular tufts there are low numbers of monocytes containing high numbers of Cytauxzoon felis schizonts. (HE 400X).

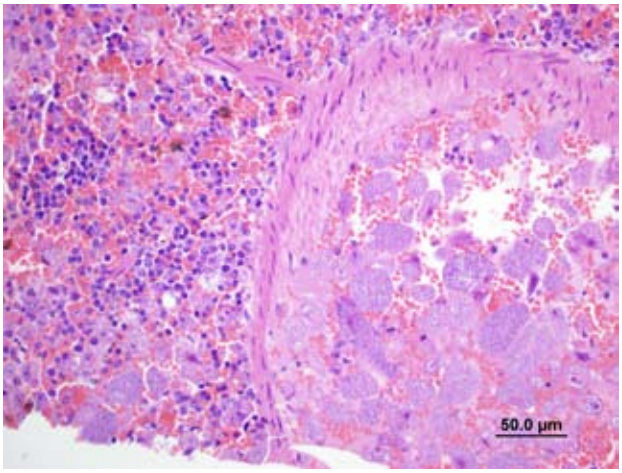
Dermacenter variabilis.[4]

The infection is associated with both tissue (schizogonous) phases and an intraerythrocytic phase that correlates with the clinical phases of severe circulatory impairment and hemolytic anemia, respectively.[1] The clinical signs in domestic cats include depression, anorexia, pyrexia, dehydration, pallor, icterus, dark urine and occasionally dyspnea.[1,3]

The cat in the current case presented to the veterinarian, after partial abortion (aborting three of six fetuses) the previous night, in a near comatose stage with lethargy and mild gingival icterus. Initially, the cat had a reduced heart rate, which progressed to seizures, then death. A



2-3. Cat, liver. Within the hepatic vessels and hepatic sinusoids there are moderate numbers of monocytes containing high numbers of Cytauxzoon felis schizonts. Photomicrograph courtesy of University of Georgia, Tifton Veterinary Diagnostic and Investigational Laboratory, 43 Brighton Road, P.O. Box 1389, Tifton, GA 31793, USA.



2-4. Cat, liver. Hepatic vessels are occluded by high numbers of monocytes containing numerous Cytauxzoon felis schizonts Photomicrograph courtesy of University of Georgia, Tifton Veterinary Diagnostic and Investigational Laboratory, 43 Brighton Road, P.O. Box 1389, Tifton, GA 31793, USA.

blood smear was collected immediately postmortem by cardiac puncture. Numerous schizont-laden macrophages and small round ring-shaped piroplasms in several erythrocytes were observed in the Wright-Giemsa stained smears. Generally, occasional schizont-laden macrophages may be observed on the feathered edge of blood smears in infected cats, but numbers similar to those in this case are unusual. Large numbers of schizont-laden macrophages in blood smear in the current case could be attributed to collection of blood from the heart. Because of their large size these cells are less likely to be found in peripheral circulation and are not normally found in blood smears from infected cats.[6] As in the current case, it was previously reported that high percentage of parasites are identified histologically from the spleen, liver, or lungs, suggesting sampling from these organs as the most appropriate sites for organism identification.[4] Because the tissue phase occurs prior to the erythrocytic phase, some cats can be severely ill but not have detectable parasites in their red blood cells.[1]

Three remaining fetuses were recovered at necropsy. Formalin fixed tissues from the cat and some fetal tissues (skeletal muscle, developing bone and placenta) were examined. Macrophages or piroplasms associated with the organism were not seen in fetal tissues. Whether *Cytauxzoon* infection in this cat would be incriminated for the partial abortion observed could not be ruled out.[6] The tissue schizonts are the phase that is responsible for clinical manifestation of cytauxzoonosis.[4] Clinical *Cytauxzoon felis* infection is usually fatal in domestic cats [1,4], although some infected cats survive.[1] Because of this, domestic cats (*Felis domesticus*) are regarded as accidental hosts.[6] Bobcats, thought to be the reservoir hosts, are persistently parasitemic, yet they rarely manifest marked clinical disease.[4] Rare fatal cases of cytauxzoonosis in free-ranging bobcats has been reported.[5]

AFIP Diagnosis: Kidney, liver, and spleen: Histiocytosis, intravascular, diffuse, moderate with intrahistiocytic schizonts, etiology consistent with *Cytauxzoon felis*

Conference Comment: The contributor gave a good overview of *Cytauxzoon felis* and its importance in the domestic cat. During the conference other blood parasites were discussed. With erythrocytic parasites, it is important to determine the exact location of the parasite within the erythrocyte in order to accurately identify the parasite and thus make a correct diagnosis. Included below is a non-comprehensive list of blood parasites that are important in veterinary medicine.

Location	Parasite	Hosts
Intracellular parasites (within erythrocytes)	*Hemoproteus spp.* *Leukocytozoon spp.* *Plasmodium spp.*	Birds
	Cytauxzoon felis *Babesia cati* *Babesia felis*	Cats
	Anaplasma marginale *Anaplasma centrale*	Cattle
	Babesia bovis *Babesia bigemina*	Cattle
	Theileria mutans *Theileria annulata*	Cattle
	Theileria cervi	Deer, Elk
	Babesia canis *Babesia gibsoni*	Dogs
	Babesia equi *Babesia caballi*	Horses
	Babesia ovis *Babesia motasi*	Sheep
Epicellular parasites (On membrane surface of depression of erythrocytes)	*Trypanosoma johnbakeri*	Birds
	Hemobartonella felis (Mycoplasma haemofelis)	Cats
Epicellular parasites (On membrane surface of depression of erythrocytes)	*Hemobartonella canis (Mycoplasma haemocanis)*	Dogs
	Eperythrozoon suis (Mycoplasma haemosuis)	Pigs
	Eperythrozoon wenyoni	Cattle
	Eperythrozoon sp.	Llamas

Extracellular parasites (within the plasma)	*Dipetalonema reconditum*	Dogs
	Dirofilaria immitis	Dogs (sometimes cats)
	Setaria spp.	Horses
	Trypanosoma theileri	Cattle
	Trypanosoma congolense	
	Trypanosoma vivax	Dogs
	Trypanosoma cruzi	Horses
	Trypanosoma brucei	
	Trypanosoma evansi	

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Contributing Institution: University of Georgia, College of Veterinary Medicine, Tifton Veterinary Diagnostic and Investigational Laboratory, 43 Brighton Road, (P.O. Box 1389), Tifton, GA 31793, USA

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CASE III – G08-015746 (AFIP 3106209)

Signalment: Adult, female, *Chinchilla lanigera*, Chinchilla

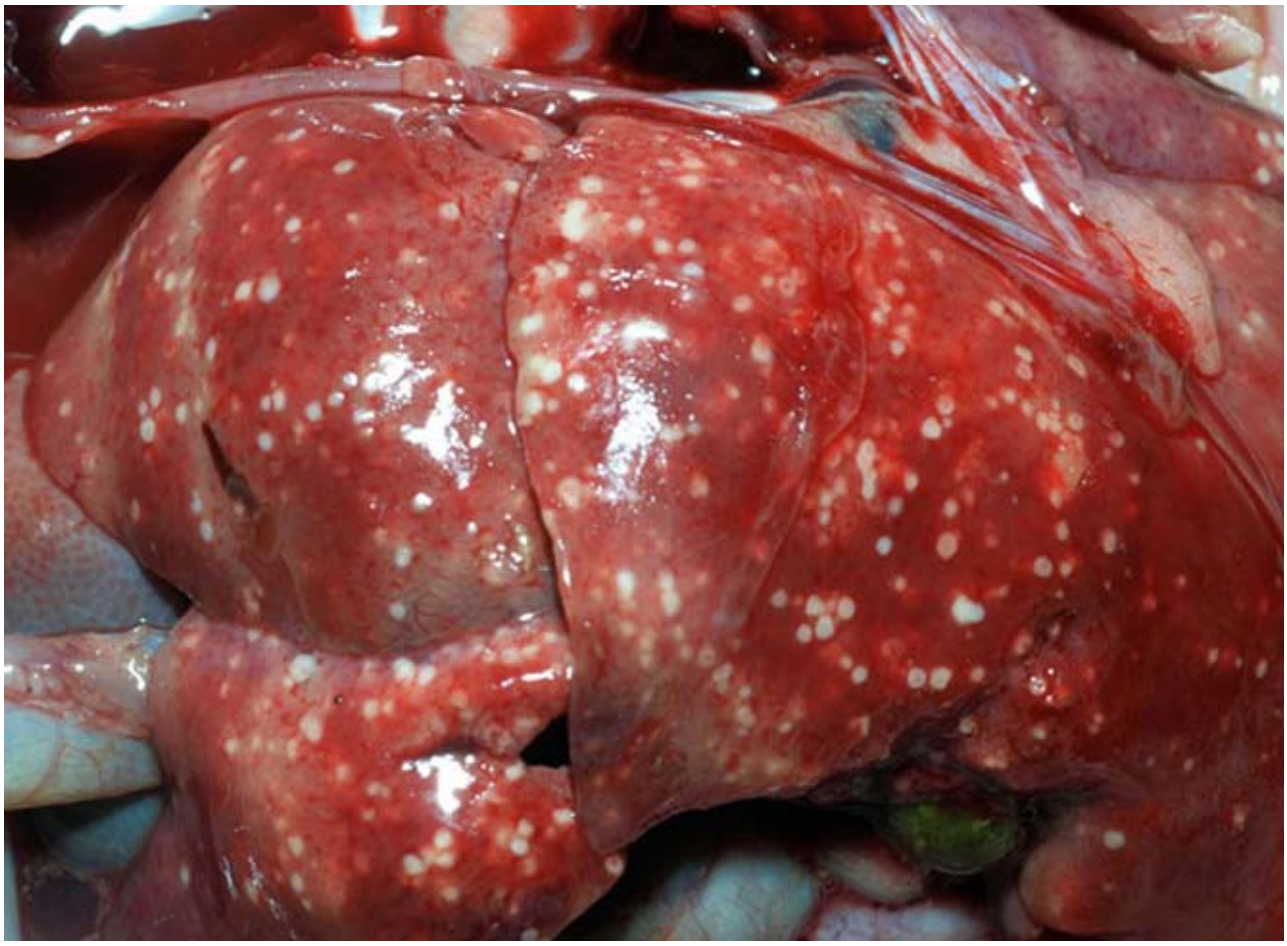
History: 14 of 100 adult fur-ranched chinchillas died over a one-week period. Clinical signs prior to death include listlessness, loss of balance, ataxia.

Gross Pathology: Six animals were submitted for postmortem, all with similar gross lesions including multiple random 1-4 mm white foci visible on the capsular surface and throughout the parenchyma of the liver **(Fig. 3-1)**; similar foci were visible through the serosa of the small intestine, cecum and colon **(Fig. 3-2)**. Cecal and colonic content was dry and inspissated.

Laboratory Results: Listeria monocytogenes was isolated from lung (1+) and liver (4+).

Histopathologic Description: Scattered throughout the liver are random multifocal areas of parenchymal necrosis and inflammation, characterized by a central zone of cellular debris, fibrin exudation and marked neutrophil infiltration, with scattered aggregates of small rod-shaped bacteria **(Fig. 3-3)**; lesser numbers of lymphocytes and histiocytes are evident around the periphery of some foci. Moderate numbers of hepatocytes, predominantly in zones 2 and 3, have one or more large clear cytoplasmic vacuoles (lipid), and there are occasional fibrin thrombi in hepatic vessels. Tissue gram stains (Brown and Brenn) reveal large numbers of gram-positive rod-shaped bacteria in the foci of necrosis **(Fig. 3-4)**.

Similar foci of mucosal to transmural necrosis are evident in sections of small intestine, cecum and colon, and there is fibrinosuppurative meningitis evident in sections of



3-1. Chinchilla, liver. Multiple random white foci visible on the capsular surface of the liver. Photograph courtesy of Animal Health Laboratory, University of Guelph, P.O. Box 3612 Guelph, Ontario CANADA N1H 6R8.

brain (tissues not shown).

Contributor's Morphologic Diagnosis: Multifocal random hepatic necrosis and microabscessation with intralesional gram-positive rods, compatible with *Listeria monocytogenes* infection

Contributor's Comment: Listeriosis is a common disease of ranched chinchillas [2,6] first reported by MacKay et al. in 1949.(4) The disease is caused by *Listeria monocytogenes*, a facultatively anaerobic, gram-positive, rod-shaped bacterium that is considered part of the normal microbial flora in ruminants, and can persist in the environment as a plant saprophyte on decaying vegetation.[1] Listeria is also a potential pathogen, capable of causing three distinct clinical forms of disease: septicemia (principally in monogastric animals), encephalitis or abortion (principally in adult ruminants). While a wide range of animal species can be affected, chinchillas are considered highly susceptible to the visceral septicemic form of disease. Ingestion of contaminated food (such as pellets or hay contaminated with rodent, chicken or ruminant feces) can result in intestinal infection, with bloodborne dissemination to liver causing multifocal necrosis and abscessation, with subsequent septicemia and systemic spread to various organs including lymph nodes, lung, spleen, and brain.

AFIP Diagnosis: 1) Liver: Hepatitis, necro-suppurative, multifocal, moderate, with numerous bacilli, Chinchilla (*Chinchilla lanigera*), rodent
2) Liver, hepatocytes: Vacuolar change, lipid-type, diffuse, mild

Conference Comment: *Listeria monocytogenes* is an important pathogen of not only animals, but also humans, and is well known for its ability to grow in a wide temperature range to include refrigerator temperatures.

3-2. Chinchilla, liver. Similar scattered white foci visible through the serosal surface of the cecum. Photograph courtesy of Animal Health Laboratory, University of Guelph P.O. Box 3612 Guelph, Ontario CANADA N1H 6R8.

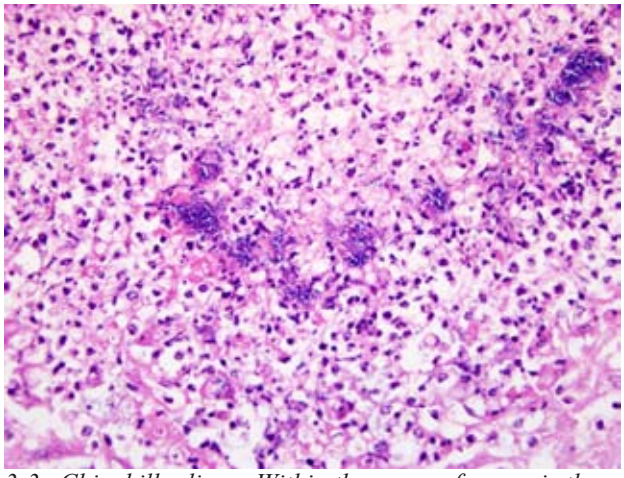
Because of this ability, outbreaks of food-borne listeriosis occur periodically in the United States. In veterinary medicine, it may be most notorious for its ability to grow in farm feeds, specifically silage, and cause subsequent clinical disease in cattle unlucky enough to be fed this tainted meal.[3]

L. monocytogenes produces a hemolytic toxin that adds to its virulence which distinguishes it from other species that are nonpathogenic. *L. monocytogenes* can also survive within macrophages and thus hide from the host immune response. A strong cell-mediated immune response by the host is essential to prevent development of clinical listeriosis.[3]

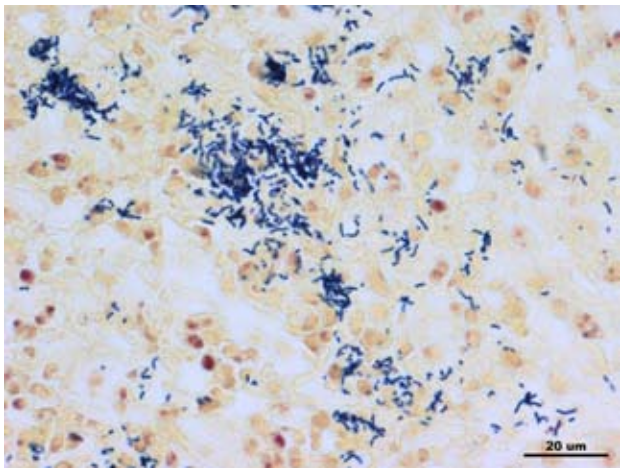
The three clinical syndromes of listeriosis—septicemia, abortion, and encephalitis— rarely overlap, thus suggesting a separate pathogenesis for each. The abortion syndrome usually occurs in ruminants in late gestation and probably gains access to the uterus and fetus via the bloodstream. The septicemic form has been reported in calves, foals, and aborted fetuses and manifests as numerous miliary microabscesses in the liver and to a lesser extent in other organs. In the encephalitic form, contaminated silage is fed to ruminants and the organism invades the oral mucosa through abrasions and enters the trigeminal nerve. The bacteria use the axon as a highway to gain entry to the brain. Listeria monocytogenes has a predilection for the brainstem and causes microabscessation that can sometimes be seen grossly. Histologically, these areas are characterized by either small aggregates of neutrophils, or more commonly small glial nodules are observed.[5]

Other domestic species rarely get listeriosis, but when they do the clinical signs and disease progression follow a similar pattern.[5]

Contributing Institution: Animal Health Laboratory,



3-3. Chinchilla, liver. Within the areas of necrosis there are numerous intrahistiocytic and extracellular bacilli. (HE 400X).



3-4. Chinchilla, liver. Within necrotic areas there are numerous gram positive bacilli (B&B). Photomicrograph courtesy of Animal Health Laboratory, University of Guelph P.O. Box 3612 Guelph, Ontario CANADA N1H 6R8.

University of Guelph, Guelph, Ontario, Canada http://ahl.uoguelph.ca

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CASE IV – 07N 929 (AFIP 3105935)

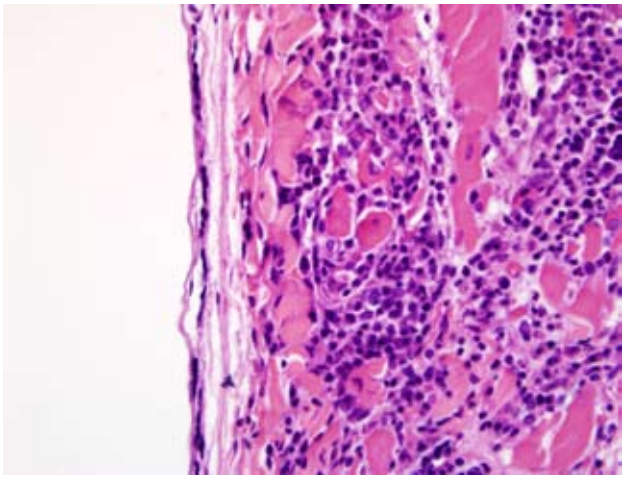
Signalment: 2.5-year-old, intact male mixed breed dog, *Canis lupus familiaris*, canine

History: Shortly before death, the dog was apparently clinically normal. He was let outside for 15 minutes, and was subsequently found dead in the owner's back yard.

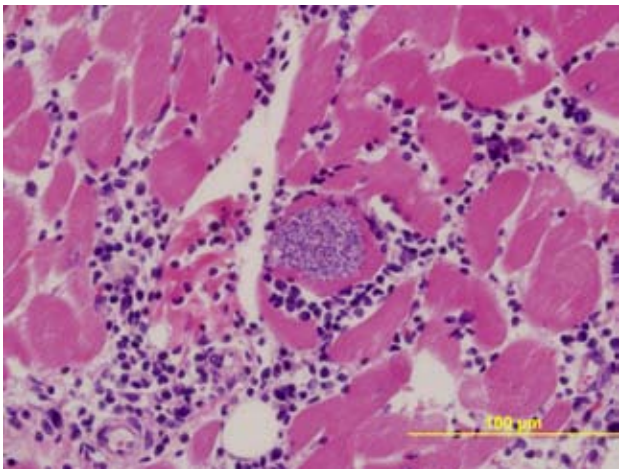
Gross Pathology: The gums were ashen blue with a line of redness along the periodontal margin. There were 30-50 white adult filarid nematodes consistent with *Dirofilaria immitus* in the right ventricle, right atrium, caudal vena cava, and main pulmonary artery. The right ventricle appeared mildly enlarged and dilated. The lungs were moist and diffusely dark red. On sectioning, the lungs effused copious amounts of blood. The margins of the liver were slightly rounded and it also effused blood on sectioning. No other pathologically relevant lesions were detected.

Laboratory Results: NA

Histopathologic Description: Heart: There were marked infiltrates of lymphocytes, plasma cells and occasional histiocytes throughout the myocardial



4-1. Dog, heart. Multifocally, infiltrating and replacing the myocardium are moderate numbers of lymphocytes, plasma cell and fewer histiocytes which often separate and surround necrotic cardiac myocytes. Necrotic cardiac myocytes are shrunken, brightly eosinophilic, fragmented sarcoplasm that has lost cross striations and contains pyknotic nuclei. (HE 200X).



4-2. Dog, heart. Cardiac myocyte containing amastigotes and adjacent necrotic cardiac myocyte. Photomicrograph courtesy of Department of Patho-biological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

endomysium in areas of myocardiocyte degeneration, fragmentation, necrosis, and loss **(Fig. 4-1)**. Occasional myocardiocytes have sarcoplasmic pseudocysts containing approximately 2-4 um diameter oblong amastigotes **(Fig. 4-2)** consistent with *Trypanosoma cruzi* (some slides may not have the organism). At 1000x magnification, amastigotes have both a basophilic round nucleus and smaller basophilic linear kinetoplast. Specific immunohistochemical stains confirm the organism's identity as *Trypanosoma cruzi* **(Fig. 4-3)**.

Contributor's Morphologic Diagnosis:
Heart: Severe, multifocal to coalescing, lymphoplasmacytic and histiocytic myocarditis with intralesional myocardiocyte cytoplasmic amastigotes consistent with *Trypanosoma cruzi*

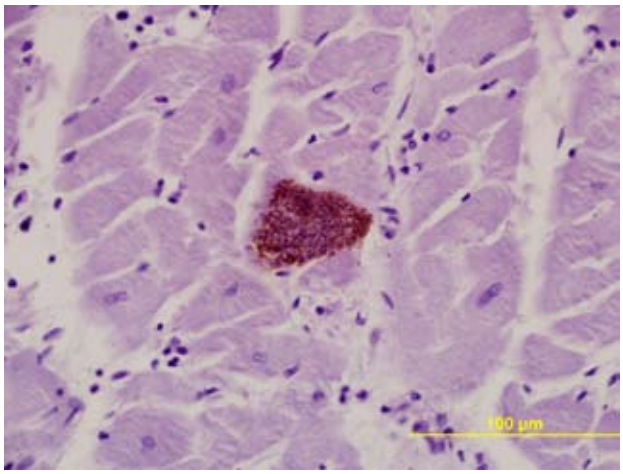
Contributor's Comment: Differential diagnoses for lymphoplasmacytic and histiocytic myocarditis in dogs include a long list of potential infectious and parasitic pathogens. Viral pathogens include canine parvovirus 2, canine morbillivirus, canine and porcine herpesviruses, and West Nile Virus.[11] Canine morbillivirus is more likely to cause degenerative changes than the mononuclear inflammation seen in this case.

Bacterial causes can include a multitude of organisms which may eventually cause chronic mononuclear cell infiltration and myocardial degeneration; however, in most cases there is a significant suppurative component. Some of the more commonly reported organisms include *Citrobacter* spp. and *Bartonella* spp. [4,6] *Borellia burdorferi* is the most commonly reported spirochete capable of causing myocarditis in dogs.

Fungal myocarditis has been reported in dogs associated with *Cryptococcus neoformans, Coccidioides immitis, Blastomyces dermatidis*, and *Aspergillus terreus*. Parasitic causes of myocarditis are also frequently reported and include *Angiostrogylus vasorum* (an eosinophilic component would be expected), and numerous protozoa. With the lymphoplasmacytic and histiocytic inflammation seen in this case, protozoal myocarditis was considered the top differential. Reported causes of protozoal myocarditis include T*oxoplasma gondii, Hepatozoan canis, Neospora canis, Trypanosoma cruzi*, and *Trypanosoma brucei*.

Immunohistochemical stains are available for the aforementioned protozoa. The morphology of protozoal amastigotes (with prominent kinetoplasts), intracardiomyocyte pseudocyst formation, and positive immunohistochemical staining for *T. cruzi* led to diagnosis of trypanosomiasis in this case.

Chagas disease is endemic to the southeastern United States and is considered an important differential for dogs with sudden death.[10] Widespread myocarditis can result in potentially fatal arrhythmias and/or cardiac contractile



4-3. Dog, heart. Immunohistochemical stain for Trypanosoma cruzi *demonstrating diffuse immunopositivity. Photomicrograph courtesy of Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.*

failure which can lead to peracute heart failure. One of the interesting features of the myocarditis and myocardial degeneration/necrosis seen in Chagas disease is that the most intense areas of inflammation and cardiomyocyte change are often unassociated with the parasite. Several studies have looked at the immunopathogenesis of Chagas disease and conclude that myocarditis may be a result of cell-mediated immune and/or autoimmune responses along with a microangiopathy.[1,2,5,9]

AFIP Diagnosis: Heart: Myocarditis, lymphoplasmacytic, multifocal, moderate with rare sarcoplasmic pseudocysts containing numerous amastigotes, etiology consistent with *Trypanosoma cruzi*

Conference Comment: There was substantial slide variation in this case, and some slides did not contain the parasite. This fact was duly noted during the selection process, but this case was used because it is an excellent representation of the inflammatory lesion and contained excellent contributor comments. The goal for the attendees was to provide a comprehensive histologic description leading to an acceptable and defensible differential diagnosis, which this case provided.

T. cruzi, the cause of Chagas disease, is an extremely important disease in not only the southern United States, but also throughout Central and South America. It has been estimated that up to 10 million people in Central and South America have Chagas, a majority of which are unaware they are infected. Chagas disease is spread by insects from the *Triatomidae* family. These insects are also known as "assassin bugs" or "kissing bugs." These bugs like to hide in the walls of mud huts to emerge at night for a blood meal. They feed near the oral or ocular mucous membranes, ingest a blood meal, and simultaneously defecate on their unfortunate victim. The person or animal bitten will scratch this area and introduce the trypomastigote form of *T. cruzi* into a mucous membrane or wound, and the organism enters the host. The trypomastigotes follow the blood stream to the heart, where they enter a cell and become amastigotes. This form multiplies by binary fission. Pseudocysts can be seen in cardiac myocytes at this stage. These cysts eventually rupture, releasing trypomastigotes. Trypomastigotes are taken up in the blood by the kissing bug, and in the intestinal tract the organism transforms into the epimastigote form. Once the organism travels to the rectal mucosa of the kissing bug, transformation back to the trypomastigote form occurs and the cycle continues.[3,7,8,9,12]

There is an acute phase of Chagas disease, characterized by nonspecific signs including fever, fatigue, diarrhea, and vomiting. Romana's sign, a characteristic swelling around the eye near the bite wound, is well recognized manifestation of this disease. The vast majority of people recover in 4-8 weeks. Several years later, a chronic phase develops in around 30% of those infected and manifests most commonly as a cardiomyopathy, or problems with the gastrointestinal system to include megaesophagus and megacolon. Less commonly it causes neurologic disease.[3,12]

Trypanosoma cruzi is a kinetoplastid, intracellular protozoan parasite. Ultrastructurally, the kineto-plast, which is a DNA-containing cytoplasmic organelle, can be used to identify protozoans. In *Trypanosoma* infections, the amastigotes have a kinetoplast that is parallel to the nucleus, whereas in *Leishmania*, the kinetoplast is smaller and is perpendicular to the nucleus. Other protozoans found in the heart, such as *Toxoplasma* and *Neospora*, do not have kinetoplasts.[7]

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