

**The Armed Forces Institute of Pathology
Department of Veterinary Pathology
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Conference Moderator:

Dr. Bruce Williams, DVM, Diplomate ACVP

CASE I: X26880.08 (AFIP 3134058).

Signalment: 7-month-old, castrated male, domestic ferret (*Mustela putorius furo*).

History: A ferret that was previously vaccinated with distemper and rabies vaccines was obtained at 5 months of age. A few days after re-immunization in the right hind leg the ferret appeared lethargic and sore in that leg. Over the next few weeks the lethargy continued and the soreness of the right hind leg progressed to hind end weakness accompanied by a fever (40.1 degrees Celsius), and then the hind end weakness appeared to resolve. A complete blood count during this time revealed leukocytosis characterized by a mature neutrophilia. Despite apparent resolution of the right hind leg lameness, the ferret continued to be lethargic and unthrifty, was unkempt, failed to gain weight (appeared small for his age), and was febrile (temperature 40.7 degrees Celsius). A month after the onset of clinical signs, the ferret was humanely euthanized. Terminal blood samples were collected at the time of euthanasia for complete blood count and serum biochemistry (see below). Tissues were collected and submitted for histopathologic evaluation.

Gross Pathology: The gross postmortem examination findings reported by the submitting veterinarian indicated that the ferret was in lean body condition. Significant gross abnormalities that were recorded include: roughening and thickening of the esophagus, locally extensive pallor of the musculature of the left hind leg, focal thickening of the pericardial sac and splenomegaly.

Laboratory Results:

Hematology	Reference ranges*	WBC Diff. (%) (x10E9/L)	Reference ranges* (%)
WBC 19.7 x10E9/L	7.7 – 15.4 x10E9/L	Segs 49% 9.653	24 – 78%
RBC 6.33 x10E12/L	7.3 – 12.18 x10E12/L	Bands 1% 0.197	0 – 2.2%
HGB 98 g/L	120 – 182 g/L	Eos 7% 1.379	0 – 7%
HCT 0.266 L/L	0.36 – 0.61 L/L	Lymph 40% 7.880	28 – 69%
		Mono 3% 0.591	3.4 – 8.2%
Platelets: Clumped			
		Corr. WBC 19.7 x10E9/L	
RBC Morphology			
Anisocytosis 1+			

Biochemistry Results	Reference ranges*	Biochemistry Results	Reference ranges*
Sodium 145 mmol/L	146 – 160	Amylase 9 U/L	
Potassium 5.4 mmol/L	4.3 – 5.3	Alk.Phos. 9 U/L	30 – 120
Na:K Ratio 27		CK 110 U/L	
Chloride 117 mmol/L	102 – 121	AST [GOT] 63 U/L	

Calcium	2.12 mmol/L	2.15 – 2.62	ALT [GPT]	67 U/L	82 – 289
Phosph.	1.83 mmol/L	1.81 – 2.81	Gamma-GT	0 U/L	
Urea	4.8 mmol/L	4.28 – 15.35	T. Prot.	53 g/L	
Creat.	16 umol/L	17 – 53	Albumin	21 g/L	
Glucose	7.3 mmol/L	3.47 – 7.44	Globulin	32 g/L	
Cholest.	4.29 mmol/L	1.65 – 7.64	A:G Ratio:	0.66	
T. Bili.	1 umol/L	<17	Lipase	278 U/L	

*Note: Data adapted from Lewington JH: Ferret Husbandry, Medicine, and Surgery, 2nd ed., pp. 274-275. Elsevier Saunders, Edinburgh, New York, 2007

Histopathologic Description: Esophagus: The muscular wall of the esophagus is circumferentially and diffusely infiltrated and expanded by dissecting infiltrates of large to very large numbers of neutrophils admixed with moderate numbers of epithelioid macrophages, occasional scattered small lymphocytes and plasma cells, and rare eosinophils and multinucleate giant cells. The suppurative to pyogranulomatous inflammation dissects around the circular and longitudinal layers of the muscularis externa (perifascicular myositis) and infiltrates the reticular connective tissue between individual myofibers, with disruption of the normal architecture and layering of the muscular wall. Myofibers often appear reduced in diameter (myofiber atrophy) with rare degenerative myofibers scattered within the inflamed esophageal wall. The inflammation also multifocally dissects along the connective tissues into the adjacent adventitial connective tissue and the overlying submucosal connective tissue up to the level of the muscularis mucosae, but overlying mucosal lamina propria and mucosal epithelium are spared (mural esophagitis). The small-caliber blood vessels within the inflamed wall are diffusely congested and lined by plump (reactive) endothelium.

Contributor's Morphologic Diagnosis: Esophagus: Moderate to severe, diffuse, circumferential, suppurative to pyogranulomatous mural esophagitis.

Contributor's Comment: The inflammatory myopathies are a group of acquired diseases characterized by an inflammatory infiltrate of the skeletal muscle.(1) The three major categories of idiopathic inflammatory myopathies are polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM).(1-5,7) PM and IBM are CD8+ cytotoxicT cell-mediated diseases whereas DM is characterized by a complement-mediated microangiopathy.(1) Other less common forms include focal myositis, infectious myositis, macrophagic myositis, and inflammatory myopathy with abundant macrophages.(7) Similar to human inflammatory myopathies, canines also are affected by a heterogeneous group of disorders characterized by intramuscular inflammation. The most common inflammatory myopathies in dogs are immune-mediated masticatory myositis, a polymyositis (similar to human polymyositis), and generalized infectious myositis.(2,3,7) Extraocular myositis is a less common canine immune-mediated myositis.(6)

Myofasciitis in domestic ferrets is an idiopathic disease characterized by suppurative to pyogranulomatous myositis and fasciitis affecting skeletal, smooth, and cardiac muscle and associated fascial connective tissues.(4) As the condition is disseminated and muscle is the predominant target tissue, the terms “polymyositis” and “disseminated idiopathic myositis” have also been used in this condition. But, as the inflammation extends into the fascia and adipose tissue around muscle bundles, myofasciitis was thought to be a more appropriate morphologic descriptor for this condition.(4)

The disease affects young adults of both sexes and is clinically characterized by rapid onset of clinical signs: weakness, muscle atrophy or lethargy, high fever, neutrophilic leukocytosis, treatment failure and death (or euthanasia). The primary presenting sign may be an ambulatory problem, primarily recognized clinically in the hindlimbs.(4) Inflammation of the alimentary smooth muscle may result in clinical presentation due to various alimentary tract problems.(4) Gross lesions seen at the time of necropsy often include atrophy of skeletal muscle, red and white mottling and dilation of the esophagus, and splenomegaly. Upon histologic examination, suppurative to pyogranulomatous inflammation in the skeletal muscle and fascia of limbs, lumbar region, body wall, head, heart, and/or esophagus is seen. The extensive suppurative to pyogranulomatous esophagitis which can be diffuse and circumferential is thought to be a unique feature of this disease. Splenic enlargement is due to myeloid hyperplasia, which can also be seen in the bone marrow.(4) In this case, suppurative to pyogranulomatous esophagitis, suppurative to pyogranulomatous hind limb myositis, suppurative to pyogranulomatous periarthritis/pericarditis and splenic myeloid hyperplasia were observed.

On CBC, a neutrophilic leukocytosis, as seen in this case, may be observed.(4) Upon evaluation of serum chemistries, no elevations of creatine kinase (CK) or aspartate aminotransferase (AST), indicators of muscle damage, have been associated with the disease and elevations of these enzyme activities were not seen in this case. This may be because the inflammation displaces and results in atrophy of the muscles rather than resulting in significant myodegeneration or myonecrosis.(4) Alanine aminotransferase (ALT) may be mildly elevated in some cases, possibly due to muscular or hepatic damage, but such an elevation was not noted in this case.

The pathogenesis of ferret myofasciitis is unclear; possibilities considered include infectious or vaccine-related mechanisms of immune mediated disease. In a recent review paper, Garner et al. (2007) investigated some possible infectious etiologies including histologic and electron microscopic evaluation for viral inclusion bodies and viral particles, immunohistochemical staining for feline and ferret coronaviral antigens, and histologic examination and immunohistochemical staining for protozoal antigens (for *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis neurona*); the studies did not reveal any intralesional infectious agents.⁴ However, these negative findings do not rule out the possibility of an infectious process being involved in the etiopathogenesis of this disease.

In childhood idiopathic inflammatory myopathies it has been proposed that environmental triggers in the setting of an underlying genetic susceptibility may play a role in the etiopathogenesis of the lesions.(3) With regards to genetic susceptibility, specific HLA alleles have been found to be immunogenic risk factors for juvenile dermatomyositis in humans. In dogs, the association of distinct inflammatory myopathies with certain dog breeds suggests that genetic predispositions are involved in the development of canine inflammatory myopathies.(7) Garner et al. (2007) thought that a heritable basis for myofasciitis seemed unlikely as affected ferrets are from different breeding facilities and the disease has been seen in both the USA and The Netherlands.

An adverse vaccine reaction is also a consideration as a possible cause in this disease. All of the ferrets affected have been reported to have had a least one dose of canine distemper vaccine.(4) In humans, macrophagic myositis is a recently described condition in adults and (less frequently) children which develops focally after injection of aluminum-containing vaccines.(5,6) The conspicuous macrophages within the inflammatory lesion have intensely PAS-positive cytoplasm due to intralysosomal accumulation of aluminum hydroxide, an adjuvant used in vaccines. Difficulty in clearing aluminum from the injection site and abnormal immune response to prolonged tissue retention of aluminum hydroxide is considered to be the etiology of the lesion.(6) No evidence of similar lysosomal accumulation of aluminum hydroxide was observed upon electron microscopic evaluation of domestic ferrets with myofasciitis. If a vaccine is involved, a delayed-type immune reaction to a vaccinal substance (killed or modified infectious agent, adjuvant, residual substance, or contaminant) may be involved.(4)

In conclusion, myofasciitis in domestic ferrets is an idiopathic disease characterized by suppurative to pyogranulomatous myositis and fasciitis affecting skeletal, smooth, and cardiac muscle and associated fascia. The circumferential suppurative to pyogranulomatous esophagitis appears to be unique to this condition. The disease is unresponsive to medical treatments and appears to be uniformly fatal.

AFIP Diagnosis: Esophagus, submucosa, tunica muscularis, and serosa: Esophagitis, neutrophilic, chronic, diffuse, severe, with myofiber atrophy and adventitial and submucosal granulation tissue.

Conference Comment: The contributor provides an outstanding review of the entity. Points of discussion mentioned by the contributor and reiterated during the conference were the conspicuous absence of myocyte necrosis and degeneration, which is supported by the typical lack of elevations in CK and AST; sparing of the mucosal epithelium; and the characteristic predominance of neutrophils in the inflammatory milieu, which is unique in comparison to other inflammatory myopathies. Interestingly, while light microscopic and clinical pathology findings indicate an absence of myofiber necrosis, ultrastructural examination of affected myofibers reveals sarcoplasmic and mitochondrial swelling, disruption of myofibrils by edema, and disruption or destruction of Z bands.(4)

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CASE II: CP-08-6399-5 (AFIP 3134368).

Signalment: 4-month-old male ferret (*Mustela putorius furo*).

History: The ferret was lethargic, anorexic and had been losing weight. On physical examination the oral mucous membranes and ocular conjunctiva were yellow, indicative of jaundice.

Gross Pathology: The oral mucous membranes, ocular conjunctiva, mesentery and joints were yellow. The liver was enlarged and had a matted pattern. The gallbladder was distended and firm.

Histopathologic Description: Throughout the liver, the biliary tract is dilated and hyperplastic. In the biliary epithelium, meronts of coccidian parasites are abundant. Bile ducts are markedly dilated and contain neutrophils, cellular debris and coccidian oocytes. Portal tracts are surrounded by fibrous connective tissue with infiltrates of neutrophils, lymphocytes, plasma cells and macrophages. The gallbladder wall is markedly thickened and the epithelium is similarly affected as the bile ducts. Meronts contain at least 12 merozoites that measure about 2 microns in width and 5-6 microns in length. Oocytes are present intracellularly and in the lumen they measure approximately 10-11 microns in diameter.

Small intestine (not submitted): In the villus epithelium, meronts containing merozoites identical to those observed in the liver are abundant. Release of the merozoites into the lumen is also observed.

Contributor's Morphologic Diagnosis: Liver: Hepatobiliary coccidiosis with marked biliary hyperplasia, purulent cholangitis and portal fibrosis. Intestine: Intestinal coccidiosis.

Contributor's Comment: There is only one report in the literature of hepatic coccidiosis in ferrets.(4) In this report, the lesions were limited to the biliary tract and gallbladder with no involvement of the intestine. The organisms described in that report closely resemble those observed in this animal. This case is unique in that the coccidian organism was also observed in the intestinal tract. The morphologic features described for this organism suggest the coccidian is of the genus *Eimeria*. The hepatic coccidian in the ferret has not been classified, but it has been suggested that it appears morphologically similar to the intestinal *E. furonis*. The ferret in this case had both intestinal coccidia and hepatic coccidia. Whether these hepatic and intestinal coccidia are the same species that infected both organs or are different coccidian species is open to speculation.

AFIP Diagnosis: 1. Liver, biliary tract: Cholangitis and pericholangitis, chronic, neutrophilic and lymphoplasmacytic, diffuse, moderate to marked, with biliary hyperplasia, and numerous intraepithelial apicomplexan schizonts and gametes, and intraluminal apicomplexan oocysts.
2. Liver: Extramedullary hematopoiesis, multifocal, marked.

Conference Comment: Conference attendees found this to be an interesting case. Participants briefly discussed the Phylum Apicomplexa and the various *Eimeria* and *Isospora* species that affect animals, summarized in the table that follows.(1,3,4)

<i>Eimeria</i> and <i>Isospora</i> of Animals		
Host	Coccidian	Organ(s) Affected
Cattle	<i>E. zuernii</i>	Distal small intestine
	<i>E. bovis</i>	Distal small intestine
Sheep	<i>E. ovinoidalis</i>	Terminal ileum/ cecum and colon
	<i>E. ashata</i>	Distal small intestine
	<i>E. bakuensis</i>	Distal small intestine
	<i>E. crandallis</i>	Distal small intestine
Goats	<i>E. ninakohlyakimovae</i>	Cecum and colon
	<i>E. caprina</i>	Cecum and colon
	<i>E. christensenii</i>	Distal small intestine
Pigs	<i>Isospora suis</i> (neonatal pigs)	Distal small intestine
	<i>E. scabra</i> (weaners, growers)	Distal small intestine
	<i>E. deblickei</i> (weaners, growers)	Distal small intestine
	<i>E. spinosa</i> (weaners, growers)	Distal small intestine
Horses	<i>E. leuckarti</i>	Small intestine
Dogs	<i>C. canis</i>	Distal small intestine, large intestine
	<i>C. ohioensis</i> complex = (<i>C. burrowsi</i> , <i>C. ohioensis</i> , <i>C. neorivolta</i>)	Distal small intestine, large intestine
Cats	<i>C. felis</i>	Small intestine, large intestine
	<i>C. rivolta</i>	Small intestine, large intestine
Mice	<i>E. falciformis</i>	Colon
Rabbits	<i>E. stiedae</i>	Bile ducts
	<i>E. intestinalis</i>	Ileum, cecum
	<i>E. flavescens</i>	Ileum, cecum
Guinea pig	<i>E. caviae</i>	Large intestine
Ferret	<i>E. furonis</i> ^{1,4}	Small intestine, Bile ducts
	<i>E. ictidea</i> ^{1,4}	Small intestine
Chickens	<i>E. acervulina</i>	Small intestine
	<i>E. necatrix</i>	Small intestine
	<i>E. tenella</i>	Cecum

Morphologic features used to distinguish the various coccidia, such as *Cryptosporidium*, *Besnoitia*, *Sarcocystis*, *Toxoplasma*, *Eimeria*, and *Isospora* were also discussed. The primary characteristic used to differentiate the various genera of coccidia is the structure of the sporulated oocyst, particularly the number of sporocysts and sporozoites present, as summarized below.(2)

Apicomplexa Sporocyst and Sporozoite Numbers		
Coccidian	Sporocysts	Sporozoites
<i>Cryptosporidium</i>	0	4
<i>Besnoitia</i> <i>Isospora</i> <i>Sarcocystis</i> <i>Toxoplasma</i>	2	8
<i>Eimeria</i>	4	8

Additional differentiating characteristics, such as the host affected, location of the parasite within the host, and organism size and shape, were also mentioned as criteria to be considered when speciating coccidia. The moderator noted that *Eimeria* and *Isospora* may be also differentiated by their location of replication; *Eimeria* spp. replicate within the epithelium while *Isospora* spp. replicate within the lamina propria.

Attendees briefly reviewed the coccidian life cycle using *E. stiedae* as an example. In short, oocysts are shed in feces and sporulate. Sporulated oocysts contain four sporozoites that hatch within the intestine and then enter the liver via the portal vein. Sporozoites then penetrate into the bile epithelium and form trophozoites that undergo asexual nuclear division (schizogony). Schizogony results in the formation of schizonts that contain merozoites. The schizonts rupture, damaging the cell, and release merozoites which infect additional cells. Eventually,

merozoites form sexual stages (i.e. male microgametes and female macrogametes) which unite to form oocysts. Oocysts are released into the bile and shed into the feces and the cycle then repeats itself.

Participants discussed the similarities and dissimilarities between the coccidian in this case and *Eimeria stiedae*. Both replicate within the hepatobiliary epithelium and cause chronic cholangiohepatitis, with marked epithelial hyperplasia, bile duct reduplication, and portal fibrosis.(3) Additionally, *E. stiedae* produces long papillary fronds within the bile duct that are not present in the case of this ferret. The moderator noted that hepatobiliary coccidiosis is rare in ferrets and has only been reported in very young animals that are generally less than four months of age. The vacuolation of hepatocytes noted in this case by attendees is a very common finding in ferrets due to inanition and fatty mobilization.

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CASE III: LAPV2 (AFIP 3138334).

Signalment: 2-year-old, intact male ferret (*Mustela putorius furo*).

History: This animal was presented at consultation for a slow growing nodular mass on the right side of the trunk. The animal was doing well, with no other clinical problems.

Gross Pathology: The formalin-fixed specimen was a 15 x 15 x 10 mm mass with a smooth surface. Epidermal ulceration was present. On cut section, a white irregular firm mass was present in the dermis, extending into the subjacent adipose tissue.

Histopathologic Description: The mass is ovoid, well demarcated, unencapsulated, lying between an ulcerated epidermis (not present on all sections) and the superficial muscular layer of the skin. Two or three hair follicles are embedded within the proliferation. These are spindle to strap-cells arranged in interwoven bundles. Some of them are contiguous with the arrector pili muscle cells. They have an abundant, fibrillar or vacuolated, eosinophilic cytoplasm with indistinct borders. The nucleus is large, generally ovoid or fusiform with blunt ends and a large basophilic nucleolus. Anisocaryosis is moderate to marked and anisocytosis is moderate. Bi- or multinucleated cells are few. The mitotic index (1 to 2 mitoses per 10 high power fields) is low. Multiple lymphoplasmacytic inflammatory foci, with some hemosiderophages are present in and at the periphery of the mass. On some sections, a giant cell granuloma around a fragmented hair shaft is present.

Contributor's Morphologic Diagnosis: Piloileiomyosarcoma, well differentiated, dermal, ferret (*Mustela putorius furo*).

Contributor's Comment: Originating from the arrector pili muscle, this rare tumor in all animals is now well recognized in the ferret.(1,2) The histological and cytological patterns are typical. In spite of a low mitotic index (tumor with mitotic index ≥ 2 per 10 HPF is considered to be malignant) and a moderate anisocytosis, this tumor was classified as malignant because of a frank nuclear pleomorphism. However, the complete excision of this well demarcated nodule was curative and no local recurrence occurred three months after surgery. These tumors are strongly vimentin, desmin and smooth muscle actin positive (not available in our unit), and negative for cytokeratin. (2)

AFIP Diagnosis: Haired skin and subcutis: Leiomyosarcoma, low-grade (piloleiomyosarcoma).

Conference Comment: Conference participants concurred with the contributor's diagnosis. Classification schemes in humans use mitotic rate to distinguish piloleiomyosarcoma from piloleiomyoma, with a mitotic rate of ≥ 2 per 10 HPF indicative of malignancy; the degree of nuclear pleomorphism does not correlate with malignancy in human classification schemes for this neoplasm.(2) In this case, the mitotic rate averages 1 per HPF, and there is marked anisokaryosis, as noted by the contributor. Additional microscopic findings noted by participants were follicular atrophy overlying the neoplasm with many follicles in telogen, mild superficial dermal edema, mild superficial lymphoplasmacytic dermatitis, and minimal parakeratosis.

Basal cell tumors and mast cell tumors are the most commonly reported cutaneous neoplasms in ferrets. Reports of piloleiomyosarcoma in ferrets suggest that it behaves similarly to its human equivalent, i.e., it carries a good prognosis with no reports of distant metastasis and infrequent local recurrence.(1,2) This case illustrates the potential confusion that may result from classifying a neoplasm as malignant versus benign based on histologic assessment alone, particularly when histomorphology may not be predictive of a significant difference in biological behavior.

In humans, superficial leiomyosarcoma is classified by its location and origin; the dermal variety (i.e. piloleiomyosarcoma) originates from arrector pili muscles, while its subcutaneous counterpart (i.e. angioleiomyosarcoma) originates from vascular smooth muscle. The distinction between the two variants, unlike the distinction between piloleiomyoma and piloleiomyosarcoma, is clinically significant because, while piloleiomyosarcomas have only a 30% incidence of local recurrence and no reported metastasis, angioleiomyosarcomas have a 54% incidence of local recurrence and a 39% incidence of distant metastasis.(2)

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CASE IV: TAMU 01 2009 (AFIP 3139655).

Signalment: 20-year-old Arabian mare (*Equus caballus*).

History: The mare was presented obtunded and icteric with prolonged prothrombin time (PT) and partial thromboplastin time (PTT) and markedly elevated liver enzymes (GGT, AST), ammonia, bilirubin and bile acids. The animal received tetanus antitoxin soon after she foaled approximately 60 days prior to presentation. She was anorexic and had lost weight for about a month. The mare was treated with fluids, lactulose, dextrose, metronidazole, pentoxifylline and banamine. She developed episodes of ventricular tachycardia. An ultrasound-guided liver biopsy was taken. On ultrasound, the liver appeared small. Due to the grave prognosis associated with disease progression and the results of the liver biopsy, the owner elected for humane euthanasia.

Gross Pathology: Diffusely the liver had an accentuated, lobular pattern (zonal necrosis and degeneration). The urinary bladder mucosa was uniformly red (congestion / hemorrhage). The uterus was thickened with a reddish mucosa and two, small, fluid-filled endometrial cysts measuring 1 and 0.5 cm in diameter respectively (endometrial lymphatic cysts).

Histopathologic Description: Diffusely disrupting and collapsing the hepatic lobular architecture and distorting the hepatic cords, there is marked loss of centrilobular and midzonal hepatocytes. The remaining hepatocytes have loss of cellular detail, swollen cytoplasm with multivacuolation (degeneration) or pyknotic nuclei (necrosis). Many hepatocytes contain globular to granular, irregularly-shaped, yellow-brown material (hemosiderin / bile). The hepatic sinusoids are markedly expanded by eosinophilic, homogeneous material (edema) and erythrocytes (congestion). The parenchyma is diffusely but mildly infiltrated with macrophages, neutrophils, and rare plasma cells and lymphocytes. Many Kupffer cells are distended with intracytoplasmic, dark-brown pigment (hemosiderin) and rare erythrocytes (erythrophagocytosis). The portal areas are closely approximated (lobular collapse), often with

portal-portal bridging, and are markedly expanded by proliferation of bile ducts that are lined by cuboidal to columnar cells with abundant often vacuolated cytoplasm, and a large vesicular nucleus. The cells occasionally pile up but have rare mitotic figures (biliary hyperplasia). Multifocally, the portal areas are infiltrated by fewer lymphocytes, macrophages, plasma cells, and neutrophils and rare eosinophils.

Contributor's Morphologic Diagnosis: Liver: Subacute hepatopathy with submassive hepatocytic degeneration and necrosis with lobular collapse, biliary hyperplasia, hemorrhage and mild, diffuse pericholangial hepatitis.

Contributor's Comment: Ever since its discovery by Theiler (thus the name Theiler's disease) in South Africa, this remains one of the most common causes of hepatic failure in horses. The onset of clinical signs is acute, and often, rapidly progresses to death. Occasionally, some horses survive.(4) The disease is also known as "serum hepatitis," "serum sickness," and "idiopathic acute hepatic failure." Theiler's disease is historically associated with injection of biologicals of equine origin such as tetanus antitoxin, *Clostridium perfringens* toxoids, equine herpesviral vaccine, pregnant mare serum and commercial plasma.(1,2,4,5,7) Clinically, the disease is manifested after an incubation period ranging from 40-90 days following an exposure to a biological. The clinical manifestations are reflective of underlying hepatic and central nervous system (CNS) disorders. These include icterus, mild circling to maniacal behavior, continuous walking, head pressing, blindness and ataxia. Although an association between the disease and use of a biological of equine origin is well accepted, sporadic cases without a history of biological make some veterinarians think there is an alternate cause, possibly a virus.(6) However, to date, no virus has been isolated. Macroscopically, the liver is flabby, either small or enlarged and dark-green to dark-brown. Microscopic changes invariably progress rapidly as in this case.

A liver biopsy taken 24 hours before euthanasia had expanded zones of hepatocytes arranged in ill-defined cords with a mild, interspersed, inflammatory infiltrate that followed the central veins and the portal units. The overall structural integrity was maintained. Thirty six hours later, the microarchitecture was obscured with massive drop out of hepatocytes, parenchymal collapse, poorly discernible central veins and portal triads that are variably expanded by proliferating biliary ductules, cellular infiltrates, and edema when the animal was euthanized. The ductular proliferation of cholangiolar or oval cells is a regenerative response to massive hepatocyte loss. The histologic progression was dramatic. Hepatic cord disruption and hepatocyte loss cause collapse of the lobular outline bringing the portal units closer. Histologic change in the CNS included Alzheimer type II astrocytes characteristic of hepatic encephalopathy.

AFIP Diagnosis: Liver: Hepatocellular degeneration and necrosis, massive, diffuse, severe, with intrahepatic cholestasis and hemorrhage, and multifocal moderate biliary hyperplasia.

Conference Comment: Participants briefly reviewed histomorphologic pattern recognition in hepatic lesions, emphasizing its utility in narrowing the differential diagnosis based on a sound understanding of the susceptibility of the parenchymal cells in each zone of the lobule to various insults. Specifically, centrilobular (i.e. zone 3, periacinar) hepatocytes are preferentially affected in many hepatopathies because of two key biologic features: 1) centrilobular hepatocytes are supplied by blood with the lowest concentration of oxygen in the liver, thereby making them exquisitely sensitive to hypoxic injury, and 2) they possess the greatest expression of biotransformation enzymes, including members of the cytochrome P450 superfamily (i.e. mixed-function oxidase system) responsible for most phase I biotransformation reactions that may yield transient reactive intermediates, rendering centrilobular hepatocytes highly susceptible to injury by toxins that are activated by cytochromes P450. By contrast, periportal (i.e. zone 1) hepatocytes, due to their close proximity to vascular inflow, are most susceptible to direct-acting toxicants. While the cause and pathogenesis of Theiler's disease remain enigmatic, the characteristic centrilobular to massive distribution of hepatocellular necrosis is helpful in making the diagnosis.(7)

Two noteworthy observations regarding this interesting condition were reiterated during the conference. First, while the majority of cases occur in horses that have received injections of equine-origin biologicals, many sporadic cases have occurred in horses that have not received such injections, leading to persistent suspicion of a serum-transmissible viral etiology, similar to hepatitis B in humans. Second, although rapid clinical progression typically culminating in death within 24 hours suggests an acute disease course, the microscopic appearance indicates some degree of chronicity, and acute hepatocellular necrosis and hemorrhage are not typical. Rather, there is centrilobular to massive loss of hepatocytes, macrovesicular fatty change with degeneration of most remaining hepatocytes, extensive deposition of bile pigments in Kupffer cells and hepatocytes, and mild portal fibroplasia with occasional ductular proliferation.(7)

Endogenous toxins associated with hepatic and, less commonly, renal failure may cause hepatic encephalopathy, as was diagnosed in the horse of this case. Horses are similar to humans, yet unique among domestic animal species, in that they characteristically develop Alzheimer type II astrogliosis attributable to hepatic encephalopathy, with myelin vacuolation being mild to absent. By contrast, in most domestic species the predominant histologic feature of hepatic encephalopathy is spongy vacuolation of myelin, particularly at the junction of cerebral gray and white matter and around the deep cerebellar nuclei, with Alzheimer type II astrogliosis being a variable finding.(3)

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