**Joint Pathology Center**
**Veterinary Pathology Services**

## WEDNESDAY SLIDE CONFERENCE 2018-2019

# C o n f e r e n c e 16

**16 January 2019**

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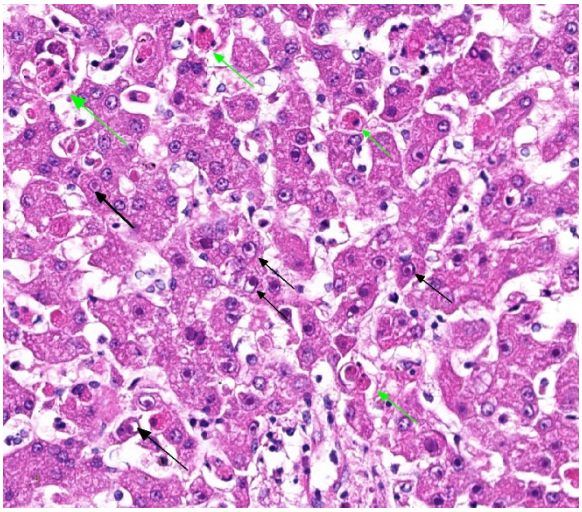
**CASE I:** FMVZ USP Case 16 (JPC 4019118).

**Signalment:** 2-month-old, female, Bernese Mountain Dog, *Canis familiaris.*

**History:** A 2-month-old female asymptomatic Bernese Mountain dog from a private kennel with sudden death few minutes after second vaccination using polyvalent vaccine (Recombitek C6/CV, Merial®) was sent to necropsy. The kennel's vaccination protocol with Recombitek was: adults, yearly; puppies, beginning at 4 weeks of age with boosters after each 2 to 4 weeks until 12 weeks of age.

**Gross Pathology:** The puppy was in regular body condition, weighing 4Kg. The lungs were diffusely marked dark red, distended, edematous; trachea's lumen contained transparent fluid. The liver was enlarged, diffuse dark red and friable. All the other organs were within normal limits of shape, size, color and consistency. The death cause being diagnosed as acute pulmonary insufficiency.



***Liver, dog. There are individualized and aggregates of shrunken, brightly eosinophilic necrotic hepatocytes, scattered randomly throughout the section (green arrows). The nuclei of numerous hepatocytes contain a 4-6um eosinophilic intranuclear inclusions surrounded by a clear halo (black arrows).***

**Laboratory results:** None.

**Microscopic Description:**
Histopathologic evaluation of the liver section showed mild disseminated periacinar (or centrilobular) necrosis and multiple midzonal foci of single-cell necrosis (apoptosis) of hepatocytes, with few neutrophils surrounding them. Numerous large eosinophilic intranuclear inclusion

bodies are present in hepatocytes and in few Kupffer's cells. Other findings include moderate congestion and diffuse mild microvesicular fatty change. The reticulin framework showed intact by reticulin stain; no changes were observed by picrossirius and Perls' stain. The microscopical evaluation of lungs reveled acute diffuse hemorrhage, congestion and edema (lesion not showed).

**Contributor's Morphologic Diagnoses:** Liver: hepatitis, acute, neutrophilic, necrotic, periacinar, disseminated, mild with eosinophilic intranuclear inclusion bodies in hepatocytes and in Kupffer's cells, etiology consistent with canine adenovirus 1, Bernese Mountain dog.

**Contributor's Comment:** Canine adenoviruses (CAV) have been pathogens of dogs. There are two types, type 1 (CAV-1) and type 2 (CAV-2), which are responsible for infectious canine hepatitis (ICH) and infectious tracheobronchitis (ITB), respectively.[2] CAV-1 causes infectious disease in dogs and other canids, has worldwide distribution and serologic homogeneity. The wild species affected are Canidae and Ursidae such as coyotes, foxes, wolves, and different species of bears. In addition, adenoviruses have been reported in otters (*Lutra lutra*) and marine mammals such as walruses (*Odobenus rosmarus*) and sea lions (*Eumetopias jubatus*)[4]. In Brazil the frequency of CAV infections in domestic dogs and wild canids is unknown.

Synonyms for ICH include epizootic fox encephalitis and Rubarth's disease and it was first recognized in dogs in 1930. CAV-1 and CAV-2, a DNA virus, are members of the genus *Mastadenovirus*, family Adenoviridae, and are antigenically and genetically closely related (75% identity at the nucleotide level). Despite of this, they are easily distinguishable by restriction endonuclease analysis and DNA hybridization. They also exhibit different hemagglutination patterns and cell tropism: CAV-1 replicates in the vascular endothelial cells, hepatic and renal parenchymal cells, whereas CAV-2 replicates in the respiratory tract and to a limited extent in the intestinal epithelia.

Transmission occurs directly by animal-to-animal contact or indirectly through exposure to infectious saliva, feces, urine, or respiratory secretions. Viral spread occurs by contact of fomites and hands. Ectoparasites can harbor CAV-1 and may be involved in the natural transmission of the disease. The incubation period in dogs is 4 to 6 days after ingestion of infectious material and 6 to 9 days after direct contact with infected dogs. Viremia lasts 4 to 8 days postinfection and a antibody response clears the virus from blood and liver by day 7 postinfection and restricts the extent of hepatic damage. However, experiments show that dogs with partial neutralizing titer by day 4 or 5 postinfection may develop chronic active hepatitis and hepatic fibrosis. The mortality rate is 10% to 30%. Coinfections with canine coronavirus, canine distemper virus, or canine parvovirus can exacerbate the disease, increasing the mortality rates.

The virus enters the host through oronasal route and initially localizes in the tonsils, where it spreads to lymph nodes and lymphatics before reaching the bloodstream through the thoracic duct.[3] The prime targets of viral localization and injury are hepatic parenchymal cells and vascular endothelial cells of many tissues, including the central nervous system (SNC). After 10 to 14 days postinoculation the virus can be found only in the kidneys and is excreted in the urine of carrier animals for up to 6 to 9 months. CAV-1 is highly resistant to environmental inactivation, surviving disinfection with chemicals such as chloroform, ether, acid,

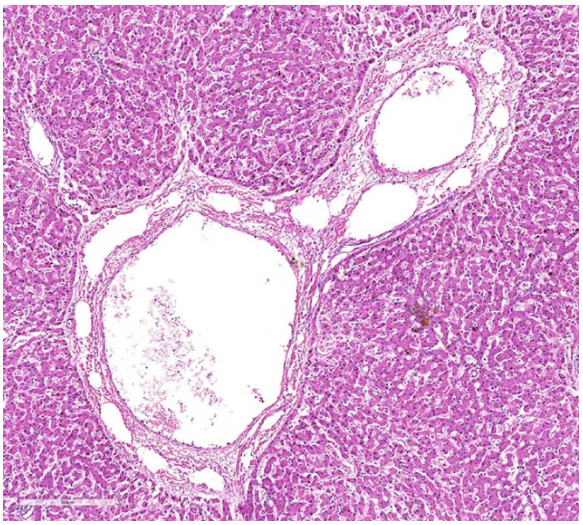
formalin and certain frequencies of ultraviolet radiation. It is inactivated after 5 minutes at 50°C to 60°C, which allows steam cleaning a mean of disinfection.[3]

Adenovirus is capable of infecting multiple organ systems; however, most infections are asymptomatic, and infections that result in disease may not be fatal. Clinical findings in the early phase of infection include increased rectal temperature (39.4°C to 41.1°C) and accelerated pulse and respiratory rates. Abdominal tenderness and hepatomegaly are usually apparent in the acutely ill dog and hemorrhagic diathesis may occur. Depression, disorientation, seizures or terminal coma can develop at any time after infection. Severely affected dogs die within a few hours after the onset of clinical signs, while mildly affected dogs may recover after the first febrile episode. Classic symptoms, such as corneal edema and anterior uveitis ("blue eye"), occur when clinical recovery begins and may be the only clinical abnormalities seen in dogs with unapparent infection. Death cause of ICH is uncertain. Although the liver is a primary site of viral injury some dogs die so suddenly that liver damage with resulting hepatic failure does not occur. Death in these dogs can result from damage to the brain, lungs, and other vital parenchymatous organs, or from the development of disseminated intravascular coagulation (DIC) during the early viremic phase of the disease.

Corneal opacity ("blue eye") and interstitial nephritis may occur 1 to 3 weeks after recovery because of deposition of immune complexes. Hematologic findings include leukopenia (<2000 cells/lL of blood; mainly attributable to a decrease in neutrophil count), increase in the serum transaminases (only in the severe forms of disease), and coagulation disorders associated with disseminated intravascular coagulation (DIC; thrombocytopenia, altered platelet formation, and prolonged prothrombin time). Proteinuria (albuminuria) can easily reach values greater than 50 mg/dL because of immunomediated glomerulonephritis. Icterus is uncommon in acute ICH, but it is found in some dogs that survive the acute fulminant phase of the disease.

Necropsy and biopsy of liver from dogs can usually confirm a diagnosis of ICH. During acute phase dogs are often in good flesh, with edema and hemorrhage of superficial lymph nodes and cervical subcutaneous tissue. Blotchy hemorrhages may be present on the serous membranes, as well as a small quantity of fluid in the abdomen. The liver is slightly enlarged, with sharp edges, turgid and friable, sometimes congested and spotted with small round areas of necrosis; the gallbladder appears thickened, edematous, and grayish or bluish white opaque in color. Edema of the gallbladder wall is a constant finding. Red strands of fibrin can be found on its capsule, especially between the lobes. Intraluminal gastrointestinal hemorrhage is a frequent finding. Gross lesions in other organs are inconstant and include multifocal hemorrhagic renal cortical infarcts, areas of pulmonary consolidation and edematous bronchial lymph nodes. The brain can be slightly swollen and on cut surface multifocal petechial hemorrhage and gray discoloration of brainstem can be observed. The Bernese puppy necropsy showed only enlarged and friable liver and edematous and dark red lungs which means that the puppy was in acute phase of CAV 1 infection.

Histopathologic changes in the liver of dogs that died of acute hepatitis include widespread centrilobular (periacinar) to panlobular necrosis and individual hepatocellular necrosis, along with neutrophilic and mononuclear cell infiltration and intranuclear inclusions in the hepatocytes



***Liver, dog. Marked dilation of lymphatics surrounding sublobular veins give a "rose window" appearance and attest to hepatic edema. (HE, 97X)***

and Kupffer's cells. Fatty changes are common but not constant. Multifocal areas of congestion, hemorrhage, and leukocyte infiltration can be observed in several organs, mainly in the lymphoid organs and kidneys, because of vascular damage and inflammation. Interstitial nephritis and iridocyclitis with corneal edema are also present in dogs recovering from ICH. Viral inclusions are initially found in the renal glomeruli and later in renal tubular vascular endothelium. Lymphoid follicles are dispersed with central areas of necrotic foci. The lungs have thickened alveoli with septal cell and peribronchial lymphoid accumulations. Alveoli in consolidated areas are filled with an exudate consisting of erythrocytes, fibrin, and fluid. Swollen, desquamated endothelial cells in meningeal vessels contain intranuclear inclusions. Mononuclear cuffing is present around small vessels throughout the parenchyma of the CNS. Mild endothelial proliferation and mononuclear perivascular infiltration persist for at least 3 weeks after clinical recovery. The histopathologic specificity of the lesions is based on the demonstration of large, intranuclear inclusion bodies. They can be also found in vascular endothelial cells and histiocytes.

The Bernese puppy had no previously symptoms and died quickly after second vaccination. The CAV 1 infection diagnosis was made based on characteristic microscopic liver lesions. The death cause was acute pulmonary insufficiency consistent with anaphylaxis, a vaccine-associated adverse event and CAV infection was considered a finding not directly correlated to dead. The lung lesions were interpreted as immediate hypersensitivity reaction to polyvalent vaccine that overlapped virus infection. Generally, in the dog, clinical signs of type I hypersensitivity include facial edema ("big head"), pruritus, hypotensive shock, weakness, dyspnea, and vomiting with or without diarrhea that can be hemorrhagic. Local or systemic reactions can occur in young puppies within 1 to 24 hours after their second or third vaccination and can result in acute clinical signs such as previously described, and death.

The ICH diagnosis can be helped by hematologic findings (eg, leukopenia, prolonged blood clotting, increased activities of alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), although the increase of transaminases is commonly observed only in severely affected or moribund dogs. Postmortem findings and histopathologic changes are highly consistent of CAV-1 infection when the *inclusion bodies,* which have been classified as Cowdry type A, are present in both ectodermal and mesodermal tissues. Confirmation of a diagnosis of ICH is obtained by virus isolation on permissive cell lines, such as Madin Darby canine kidney (MDCK) cells.[2] A polymerase chain reaction (PCR) protocol has recently been developed for molecular diagnosis. Ocular swabs, feces, and urine can be collected in vivo for virus

isolation and PCR. Postmortem samples can be withdrawn from the kidney, lung, and lymphoid tissues. The liver is rich in arginase, which inhibits viral growth in cell cultures, but it represents the most important organ for histopathologic examination. Viral growth in cells is revealed by rounding cells that form clusters and detach from the monolayer. Immunofluorescence (IF) can detect viral antigens in infected cell cultures and in acetone-fixed tissue sections or smears. Viral replication can also be demonstrated by detection of nuclear inclusion bodies in the cells after hematoxylin-eosin staining. Neither virus isolation nor IF is able to distinguish between the two adenovirus types.[2] Because CAV-2 can also be detected in the internal organs and feces of vaccinated or acutely infected dogs and CAV-1 is also frequently isolated from respiratory secretions, trachea, and lungs, distinction between CAV-1 and CAV-2 necessarily deserves laboratory examination. Restriction fragment length polymorphism analysis on viral genomes using the endonucleases PstI and HpaII generates differential patterns. Detection and differentiation of CAV-1 and CAV-2 by PCR with a single primer pair are also possible.[2] Although CAVs agglutinate erythrocytes of several species, hemagglutination is not used in routine diagnosis. Because most dogs are vaccinated and since CAV-2 infection is frequent in dogs, serology has low diagnostic relevance.

Vaccination has controlled the disease and turned it rare in domestic dog population, although severe outbreaks can be still observed in countries in which CAV vaccines are not used routinely or as a consequence of uncontrolled importation of dogs from endemic areas. Clinical management of dogs that develop ICH is primarily symptomatic and supportive expecting hepatocellular repair. Vaccination usually is repeated yearly, although after administration of two doses of CAV-2 vaccine, immunity seems to persist for more than 3 years. Even extensive vaccination has greatly reduced the incidence of CAV infections, re-emergence of ICH has been described in some countries such Italy, probably do to trading of pups with uncertain sanitary status from Eastern Europe. At the moment, there are few data on the molecular epidemiology of CAVs, but it is commonly accepted that vaccine breaks occur rarely with CAV vaccines, because the viruses are genetically stable. Accordingly, CAV infection in vaccinated dogs has been associated with maternally derived antibody (MDA) interference in the early life of the pups rather than with emergence of variants genetically distant from the prototype strains contained in CAV-2 vaccines.

We assumed first vaccination failure in the Bernese puppy due to maternally derived antibody interference. Regarding that maternally derived antibody (MDA) titers decrease below 100 around 5 to 7 weeks of age .Sporadic cases in which dogs do not get adequate vaccination during puppyhood are still seen. The duration of passively acquired immunity in the pup is dependent on the antibody concentration of the bitch. The half-life of CAV-1 antibodies is 8.6 days, and these values correlate well with the half-life for canine globulin. The recommended schedule with any vaccine for protection against ICH involves at least two doses, given 3 to 4 weeks apart, at 8 to 10 and 12 to 14 weeks of age.

The intensification of surveillance activity using new diagnostic techniques and molecular analysis tools may help to investigate the epidemiology of CAV infections more thoroughly and plan adequate measures of control in different countries.

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**JPC Diagnosis:** Liver: Hepatitis, necrotizing, multifocal, centrilobular to midzonal, mild to moderate, with edema and numerous hepatocytic and endothelial intranuclear viral inclusions.

**JPC Comment:** The contributor has provided an excellent and wide-ranging discussion of canine adenovirus-1 in the dog and in a wide range of other species.

A purported viral disease that shows a number of similarities to infectious canine hepatitis was identified in Great Britain in 1985, but may not be well-known (or even heard of by WSC participants. "Canine acidophil cell hepatitis" gets its name from the shrunken necrotic hepatocytes scattered throughout the liver, but lacks the intranuclear inclusions and CAV-1 and CAV-2 have not been identified. Transmission studies of liver homogenates induced reproducible disease, suggesting a viral agent which has not yet been characterized. Also, unlike infectious canine hepatitis, the chronic disease characterized by cirrhosis and in some cases hepatocellular carcinoma have been identified in suspect cases.[3]

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**CASE II:** 1407470-10 (JPC 4050020).

**Signalment:** 16 year old Male castrated Shetland pony

**History:** A 16 year old castrated male Shetland pony presented to Oregon State University large animal emergency service for acute onset of respiratory distress. He had an eight-month history of hind end staggering, inappetence, and difficulty maintaining weight.

On physical exam, the gelding was ataxic, dyspneic, tachycardic, tachypneic, and febrile. The complete blood count with

unremarkable. Blood gas revealed a high pCO2, bicarbonate, and lactate with low chlorine. Endoscopy revealed bilaterally closed arytenoids on inspiration. Due the history of chronic neurologic disease, plus poor condition, euthanasia was performed and the body was submitted for necropsy.

**Gross Pathology:** At necropsy, the animal was in poor body condition with no subcutaneous, visceral, or pericardial fat. Approximately 200 mL of straw colored fluid was within the thorax and 1 liter of fluid within the abdomen. The liver was markedly enlarged (2x) with swollen, rounded edges, and an irregularly nodular visceral surface. The parenchyma bulged when cut and had a prominent micronodular pattern. The right adrenal gland was swollen by a blood filled 1.5 cm diameter cyst which expanded the medullary area. A few ecchymotic hemorrhages were present on the epiglottis and in the tissue surrounding the trachea. The lungs were diffusely congested and edematous. No significant gross findings were present within the brain, spinal cord, or vertebral column.

**Laboratory results:** Following histopathology examination a frozen liver specimen was submitted for trace mineral concentration:

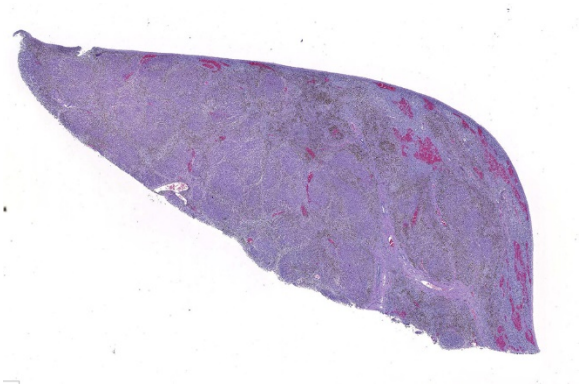
| **Mineral** | **Horse values** | **Reference Range** | **Units** |
|---|---|---|---|
| Iron | **27,048 High** | [300-900] | ug/g |
| Zinc | **81 Low** | [120-375] | ug/g |
| Copper | 25 | [12-25] | ug/g |
| Selenium | **0.5 Low** | [1.0-2.5] | ug/g |
| Cobalt | **0.12 Low** | [0.30-0.60] | ug/g |
| Molybdenum | **23.7 High** | [1.5-3.0] | ug/g |
| Manganese | 9.6 | [6.0-18.0] | ug/g |

Frozen serum was retrieved and submitted for ferritin levels at Michigan State University. A limited biochemical profile was analyzed at the OSU VDL.:

| **Mineral** | **Horse values** | **Reference Range** | **Units** |
|---|---|---|---|
| Ferritin | **46,539 High** | [43-261] | ng/mL |

**Liver chemistry profile**

| **Analyte** | **Result** | **Reference Range** | **Units** |
|---|---|---|---|
| Bun | **7 Low** | [8-23] | mg/dL |
| Total protein | 6.5 | [5.9-7.6] | g/dL |
| Albumin | 3.0 | [2.9-3.8] | g/dL |
| Total bilirubin | **2.9 High** | [0.8-2.6] | mg/dL |
| Ck | **3530 High** | [145-633] | U/L |
| Alkaline Phosphatase | **742 High** | [80-240] | U/L |
| GGT | **430 High** | [7-25] | U/L |
| AST (SGOT) | **764 High** | [212-453] | U/L |
| SDH | **>170 High** | [2.4-7.2] | U/L |



***Liver, horse. At subgross magnification, the liver parenchyma is divided into variably sized lobules by bands of dense fibrous connective tissue bridging between portal areas. There is aggregates of large amounts of a brown pigment at the periphery of the lobules (especially in subcapsular areas) and multifocal congestion and hemorrhage. (HE, 7X)***

Liver. The hepatic architecture is markedly distorted by severe fibrosis. Pseudolobules are formed from periportal bridging fibrosis with large amounts of collagen in these areas. Along the subcapsular surface, the sinusoidal patterns are disorganized. Infrequently, megalocytes are present but nuclei are generally unremarkable. An abundance of hemosiderin pigment is within stromal macrophages and Kupffer cells while hepatocytes also contain large amounts of iron positive granules. There is moderate cholangiolar hyperplasia.

The thyroid, kidney, pituitary gland, salivary gland, spleen, adrenal gland, pancreas, and colonic epithelial cells as well as mononuclear phagocytes contain the similar brown to golden pigment. Prussian blue staining of the histologic section demonstrated the pigment to be iron.

Cervical, thoracic, lumbar spinal cord (not submitted). All white matter spinal tracts, are vacuolated to some extent, with some vacuoles containing macrophages (digestion chambers). In longitudinal sections, myelin sheaths are disrupted and axons are swollen with spheroid formation.

Cerebrum (not submitted) There are single or 2-3 cell clusters of swollen astrocytes with dispersed chromatin, and scant cytoplasm (Alzheimer type II cells).

Skeletal muscle. Multifocally, the myocytes are hypereosinophilic, with internalization of nuclei and loss of cross-striations.

**Contributor's Morphologic Diagnoses:** Liver: Severe, diffuse hepatocellular degeneration and loss, with bridging portal fibrosis, marked iron accumulation, and mild biliary hyperplasia

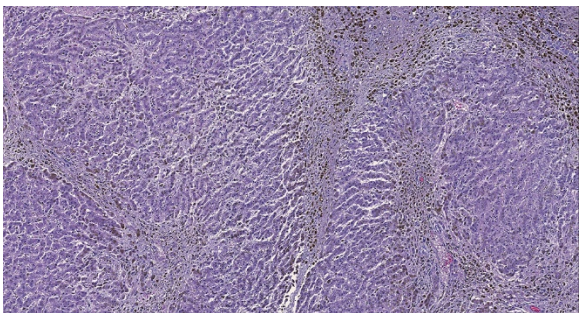
Condition: Hemochromatosis

Spinal cord: Moderate myelopathy, consistent with equine degenerative myelopathy

Cerebrum: Mild encephalopathy with Alzheimer type II cells: consistent with hepatic encephalopathy

Skeletal muscle: Mild multifocal myocyte degeneration

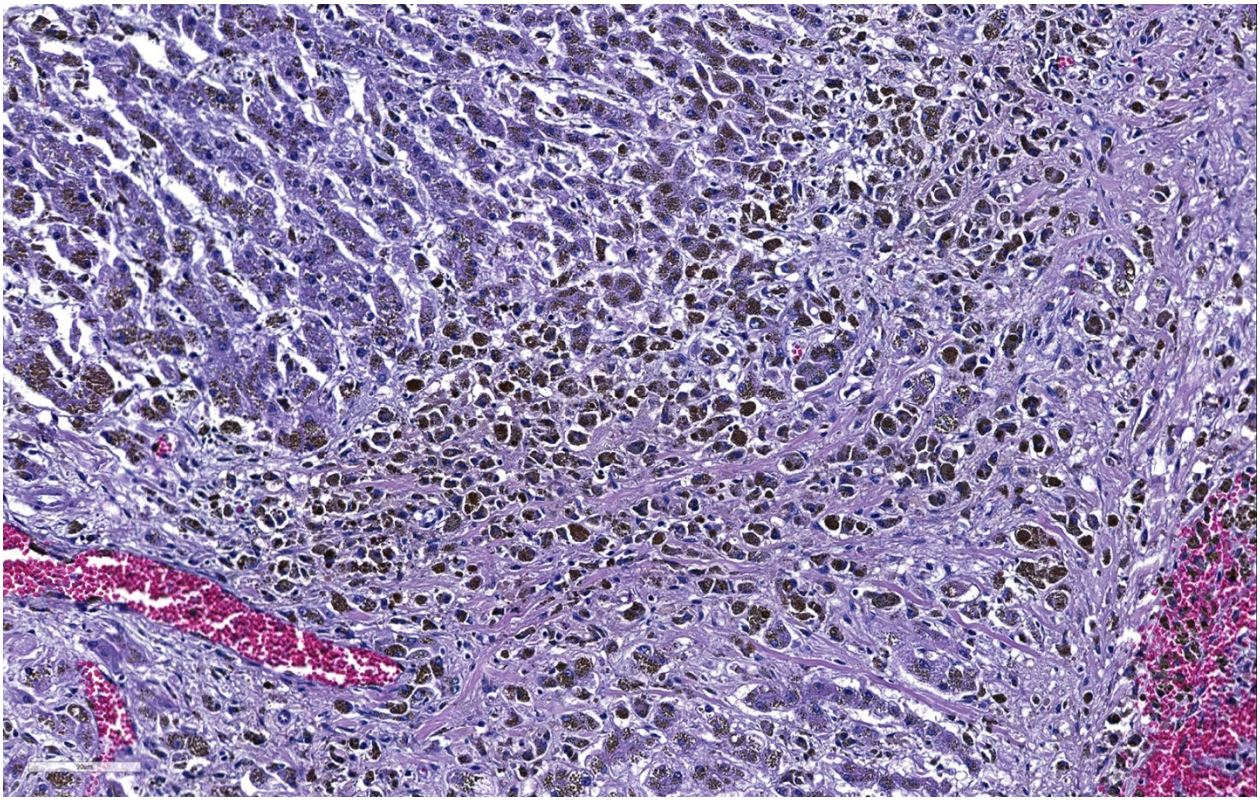
**Contributor's Comment:** Hemo-chromatosis is a well described disease affecting humans, fruit-eating and insect-



***Liver, horse. Higher magnification demonstrating irregularly lobules, bridging portal fibrosis and abundant iron pigment. (HE, 97X)***

eating birds and in Salers cattle. [2,9,10] In humans, hereditary (primary) hemochromatosis is an autosomal recessive disorder characterized by hyperabsorption of dietary iron and accelerated recycling of iron by macrophages.[1] The excessive iron is stored in the parenchymal organs, especially the liver, causing damage which may result in fibrosis, cirrhosis and hepatocellular carcinoma.[9] Mynah birds are one of the most frequently reported avian species to have hemochromatosis. Affected livers have marked chronic active cholangiohepatitis with fibrosis and iron granules within hepatocytes, Kupffer cells, and macrophages.[8] Iron is also deposited in the other organs. In this present case, Prussian blue stains of histology sections demonstrated excessive iron pigment in the thyroid gland, kidney, pituitary gland, salivary gland, spleen, adrenal glands, pancreas and colon. The serum and hepatic iron levels showed tremendous elevations.

Hemochromatosis patients and mynahs have a defect in the gene encoding for iron proteins (HFE) responsible in the iron sensing mechanism.[3,12] The mechanism is well described in humans.[10] Dietary iron is used by enteric cells in enzymatic reactions, stored as ferritin, excreted in sloughed enterocytes, or transferred to plasma. Iron absorption is mainly influenced the hepatic protein hepcidin[3]. It works by blocking ferroportin channels located in enterocytes (and macrophages), preventing iron from reaching the plasma. Dysregulation of this pathway, which is dependent on several genes, can lead to primary hemochromatosis, as low hepcidin levels allow excessive influx and storage of iron[3]. The mechanism of secondary hemochromatosis is unclear, but occurs in



***Liver, horse. Portal areas are markedly expanded by abundant collagen which extends into the periportal parenchyma entrapping individual and small groups of hepatocytes. Portal hepatocytes, entrapped hepatocytes, macrophages, and Kupffer cells contain abundant brown granular pigment. (HE, 247X)***

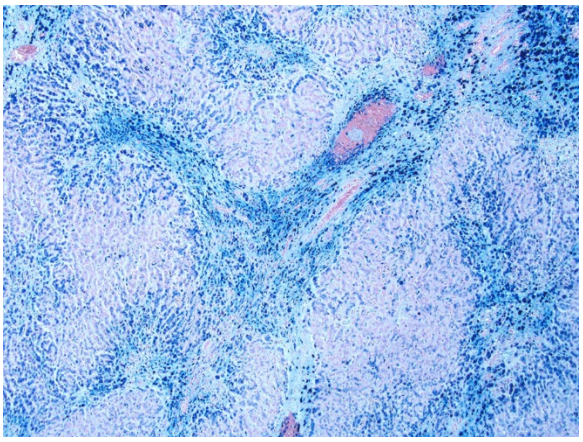
humans as a result of chronic liver disease and hemolytic anemias.[15] Untreated, hereditary hemochromatosis patients may develop severe iron overload, whereas in secondary hemochromatosis iron overload is minimal to modest. Also, the pattern of iron accumulation differs in primary and secondary hemochromatosis.[5,15] In primary hemochromatosis, iron accumulates within hepatocytes in the periportal region (acinar zone 1) whereas in secondary hemochromatosis iron accumulation is within macrophages and endothelial sinusoidal cells. The pattern observed in this case is more consistent with primary hemochromatosis.[2,15] This pony was not on any iron supplements. No other equid in this group has developed liver disease in the year since the diagnosis was made. This suggests that inappropriate intestinal iron absorption as seen with primary hemochromatosis may have been the underlying disease process in this case.

The most common signs of liver disease in horses are non-specific and include icterus, behavioral changes and weight loss.[13,15] Histopathology is vital for the definitive diagnosis of hepatic diseases. Potential causes include the acute liver disease known as serum sickness or Theiler's disease, Tyzzer's disease, cholangiohepatitis, and hepatic lipidosis.[4] Hepatotoxic disease in equids can be separated into pharmaceutical-induced (such as carbon tetrachloride, pentachlorophenols, etc) and plant/environmental exposure (pyrrolizidine alkaloidosis, blue-green algae, *Fusarium* toxicosis, etc). Iron toxicity has been reported as a cause of hepatotoxicity in orally supplemented foals.[10] Biliary calculi (choleliths), liver lobe torsion, and hepatic amyloidosis are other differentials for hepatic disease. Animals with severe destructive hepatic disease may show signs of or acute or chronic hepatic failure (wasting, jaundice) as well as neurological signs.[12] Microscopic changes of hepatic encephalopathy may include polymicrocavitation or spongiform changes of white matter (not observed in this case) and Alzheimer type II cells (present in the cerebrum). Spinal cord lesions observed in hepatic encephalopathy include vacuolation at the fasciculus proprius with no histiocytic response. This does not fit with the more widespread microscopic lesions observed in this case.

Excess iron is toxic, causing increased oxidative stress and the production of reactive oxygen species.[13] We suspect that excess iron may have promoted oxidative damage, depleting levels of antioxidants such a Vitamin E, leading to the degenerative changes to the myelinated tracks as seen in equine degenerative myelopathy. Acute onset of respiratory distress due to laryngeal and/or pharyngeal paralysis is a proposed, uncommon manifestation of hepatic disease in ponies.[13] In this instance, there is a concurrent depression in hepatic selenium levels, and high CK levels, as may be seen with nutritional myopathy, another potential cause of laryngeal malfunction in horses. Sections of heart in this pony had mild multifocal degeneration of cardiomyocytes.

**Contributing Institution:**
Oregon State University Veterinary Diagnostic Laboratory
http://vetmed.oregonstate.edu/diagnostic

**JPC Diagnosis:** Liver: Fibrosis, bridging and portal, diffuse, severe, with periportal hepatocellular degeneration and loss, edema, biliary hyperplasia and marked hepatocellular and histiocytic siderosis.



***Liver, horse. A Perl's iron stain discloses the identity and location of the pigment within hepatocytes and macrophages. (Perl's, 100X)***

**JPC Comment:** A number of mechanisms are responsible for the excessive storage of iron within the liver in man and many other species of animal. The term hemosiderosis is defined as excessive storage of iron, while the term hemochromatosis, as applied to the liver, indicates a cellular injury resulting from this storage. Primary hemochromatosis is considered to be the result of genetic mutations involving proteins regulating iron metabolism; additional information on the importance of these proteins have been studied in a series of genetic-engineered mutant mice, as well as red deer,[6] black rhinoceroses,[6] and bottle-nosed dolphins.[14]

Secondary forms of hemosiderosis result from excessive absorption of iron from sources high in iron, or may result from lack of iron chelators normally found in the diet, which allow iron normal bound in the intestinal lumen, to be freely absorbed (a mechanism which is often commonly identified as a cause of excessive liver iron in a number of zoo and wildlife species in captivity.) Affected zoo species in which hemochromatosis is routinely reported include mynah birds, toucans, birds of paradise, New World monkeys, bottle-nose dolphins and black rhinoceroses.[9]

A number of studies have been published since the submission of this case to the WSC which have helped to shed light on the pathology and potential pathogeneses of hemochromatosis in a number of species.

A 2018 case report of hemochromatosis from the University of Utrecht[16] reported a case of secondary hemochromatosis in 21 horses and one donkey housed together for a minimum of 9 years. Clinical pathology of affected animals revealed blood transferrin saturation levels of >80% and increased gamma-glutamyltransferase levels. Hepatic biopsy demonstrated excessive iron storage in hepatocytes and macrophages, hepatitis and fibrosis. Necropsy of seven animals revealed siderosis in other organs. Excessive levels of iron in drinking water was identified as a the cause, and the remaining 13 animals from 17 to 79 months post diagnosis.

A 2014 study of bottlenose dolphins resulted in the sequence of the hfe gene in this species. The hfe gene is a common cause of primary (hereditary) hemochromatosis in man. The hfe gene's product regulates the amount of hepcidin produced by hepatocytes. The point mutation of hfe in humans results in improper binding to beta2-macroglobulin preventing the transport of hfe to the hepatocyte surface, where it ultimately upregulates the expression of hepcidin. While a point mutation was not identified in affected dolphins, genomic sequencing is now a tool that may be used to pinpoint hereditary causes of hemochromatosis in animals outside the realm of human and lab animal medicine.[14]

A recent study of 83 adult Egyptian fruit bats not only documented excessive storage of iron in a number of organs including liver and spleen, but also pancreas, kidney, skeletal muscle, heart, and lung. In addition, 11

animals also had hepatocellular carcinoma, and this represents the first positive correlation between hemochromatosis and hepatocellular carcinoma in a species other than humans.[7]

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**CASE III:** 11727 (JPC 4019850).

**Signalment:** 3-years-old, female, crossbreed, heifer (*Bos taurus*)

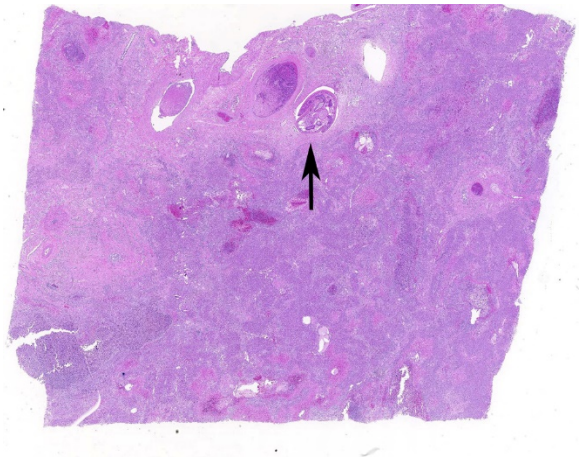
**History:** A herd of 70 heifers were in a wetland during the summer (since February 2010). These three-years-old heifers had been transferred to two years rice stubble, next to a facility to calve (August 2011). In

***Liver, ox: On cut section, the liver is fibrosis, and hemorrhageic with hemorrhagic tract and immature flukes within the parenchyma. (Photo courtesy of: Animal Pathology Department / Veterinary Diagnostic Laboratory, Veterinary Faculty; Federal University of Pelotas. 96010-900 Pelotas, RS, Brazil. http://www.ufpel.edu.br/fvet/oncovet/)***

December 2011, five animals died after 30-40 days of weight loss. The heifers had been treated with Nitroxynil in May and September 2011 before calving and with Ivermectin in October 2011 after calving. One heifer was recumbent for 12 hour and was euthanized due to unfavorable prognosis and was immediately necropsied.

**Gross Pathology:** At necropsy, mucosae showed mild anemia and jaundice. Dark brown ascitic fluid was present in abdominal cavity and there were a thick layer of fibrin among peritoneum, intestines, diaphragm and liver. The liver was enlarged with rounded edges and the capsular surface was irregular with adhered fibrin and red strips interspersed with clear areas and petechiae. Liver cut surface was irregular, friable and hemorrhagic and white channels could be observed through the parenchyma. There were some dark foci filled with debris and adult forms of *Fasciola hepatica*. The gallbladder was enlarged and the wall was thick and edematous and contained some adult forms of *F. hepatica*. Hepatic lymph nodes were enlarged and wet and fluid was drained on the cut hemorrhagic surface. Renal infarction was also observed. Fibrin and clotted blood were observed adhered to the pericardium and lung, mainly in diaphragmatic lobes.

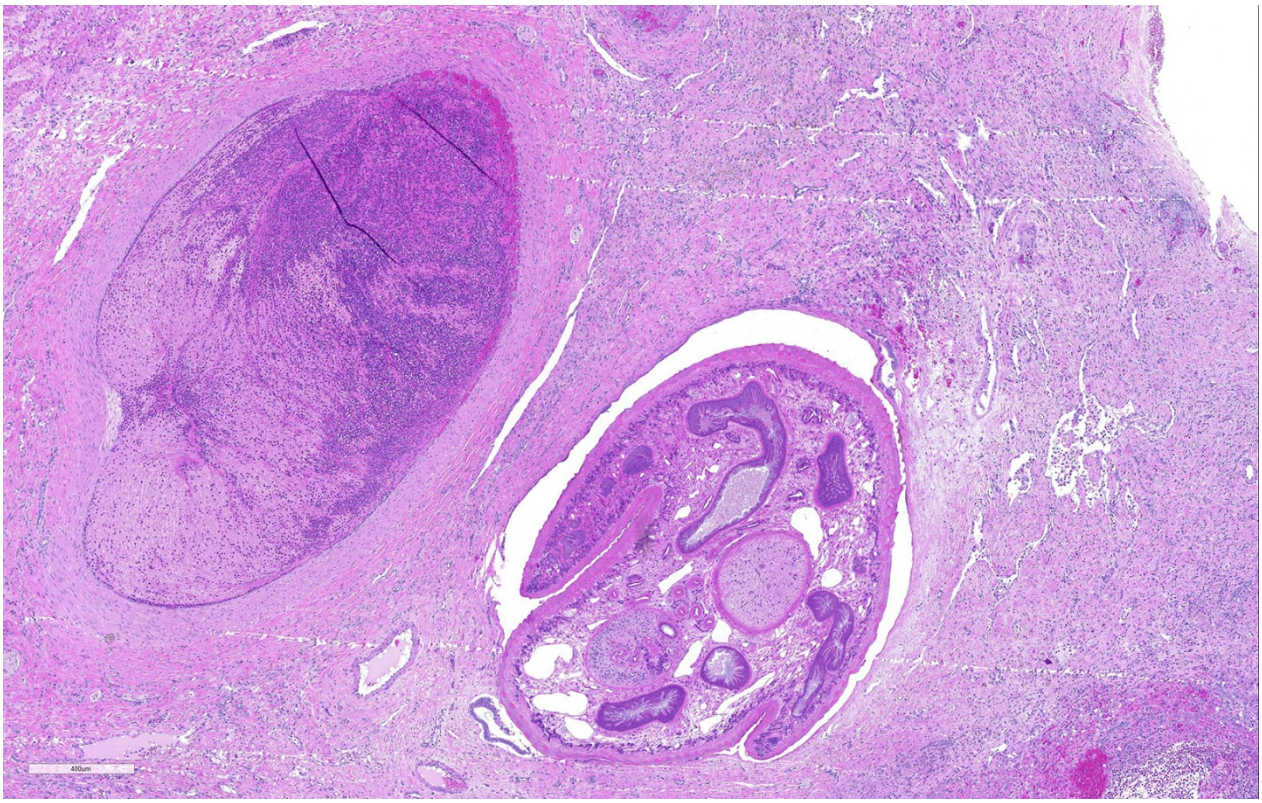
**Laboratory results:** no tests were performed



***Liver, ox: A large area of fibrosis (at top) contains a cross section of an immature trematode and several cross-sections of a thrombosed artery. Bridging portal fibrosis is evident a low magnification and at bottom areas of coagulative necrosis within several lobules. (HE, 8X)***

**Microscopic Description:**
Liver: areas of coagulative necrosis and extensive hemorrhage in streaks or foci and disruption of the parenchyma with neutrophil and eosinophil infiltration. There was also fibrosis and bili duct hyperplasia in some areas. Immature forms of *F. hepatica* were observed in the parenchyma surrounded by degenerated hepatocytes, neutrophil, eosinophil and hemorrhagia.
Additional findings (organs not submitted): peritonitis with fibrinohemorrhagic deposits on the serous surfaces of abdominal cavity. Multifocal fibrosis, hemorrhagia and neutrophils infiltration were observed in renal cortex and some hyaline casts was present in renal tubules.

**Contributor's Morphologic Diagnoses:** Hepatitis with extensive hemorrhages

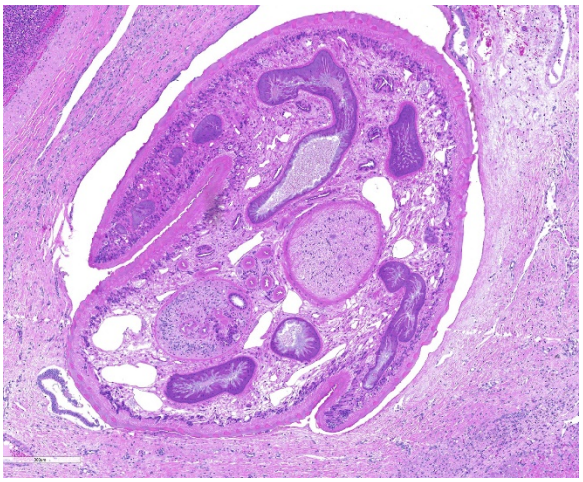


***Liver, ox: At left, a large fibrinocellular thrombus occludes a hepatic arteriole, at right is a cross section of an immature trematode. (HE, 52X)***

associated with infiltration of neutrophils and eosinophils and the presence of immature forms of *F. hepatica*. Fibrosis and bile duct hyperplasia

**Contributor's Comment:** Fasciolosis is a common parasitic disease of domestic ruminants throughout the world. The disease is caused most commonly by the trematode *Fasciola hepatica* that has a cosmopolitan distribution.[10] Liver fluke infections affect farm animals especially in terms of poor productivity, reduced milk yield and livers condemned at slaughter[11]. *F hepatica* remains an economically significant parasite of livestock and is emerging as an important zoonotic infection of humans.[6] In southern Brazil, the rate of liver condemnation at slaughter due to infection by *F. hepatica* is 10.34% for cattle and 20% for buffaloes[5], 38% for cattle and 7% for sheep.[2]

Infection occurs by ingestion; excystment occurs in the duodenum. The young flukes penetrate the intestinal wall and cross the peritoneal cavity, attaching here and there to suck blood and penetrate the liver through its capsule.[10] The acute phase follows and coincides with active liver migration of immature parasites.[6] Parasites digest hepatic tissue and cause extensive parenchymal destruction with intensive hemorrhagic lesions and immunological reactions. On the other hand, the injury of the liver can be also induced chemically by factors produced or induced by the fluke. Immature flukes wander in the liver for a month or more before settling down in the bile ducts to mature, which they do in 2-3 months.[10] The chronic phase begins 30 to 40 days after exposure as the parasites enter the bile ducts.[6]



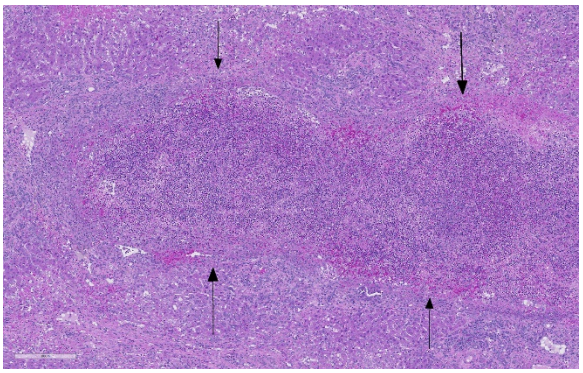
***Liver, ox: The trematode parasite has a thick cuticle with spikes, cross sections of two suckers, and numerous cross section of an intestine. The lack of a uterus, vitellarian glands and eggs indicates that this is a larval fluke. (HE, 88X)***

Usually there is no obvious reaction to the passage of young flukes through the intestinal wall and across the peritoneal cavity and parasites may be found in ascitic fluid. Acute and exudative or chronic and proliferative peritonitis occurs in heavy and repeat infections.[10]

Wet environments with temperature between 10-25°C are necessary for proliferation of the parasite as well the intermediate host (snail).[9] Precipitation may favor the accumulation of water that is a prerequisite for the disease cycle. Generally, this buildup occurs in flat or less mountainous terrain, as in Santa Vitória city (latitude S32º58'50.4"), extreme southern of Brazil, where this case occurred.

This case occurred in a recognized endemic area.[5] Acute outbreaks are not common in cattle of this region where outbreaks with high mortality are only common in sheep.[4] It seems possible that cattle from this area shows a relative resistance to the agent. A long time ago, resistance to *F. hepatica* infection in cattle has been reported.[9]

In the present case, the disease probably occurred as a result of consecutive infections since cows remained for a long period in a snail infected wetland on the farm. However, the cattle had been treated in May and



***Liver, ox: Large linear areas of lytic necrosis (migration tracts) course through the hepatic parenchyma. (HE, 70X)***

September with drugs of nitroxynil group, it is possible that these drugs cannot be effective, since does not act in the young forms of the parasite. Moreover, *F. hepatica* resistance has been reported to these drugs.[7,8] In mice secondary infections, the inflammatory response was faster and shorter. Resolution also was more rapid and often complete in 30 days.[6]

**Contributing Institution:**

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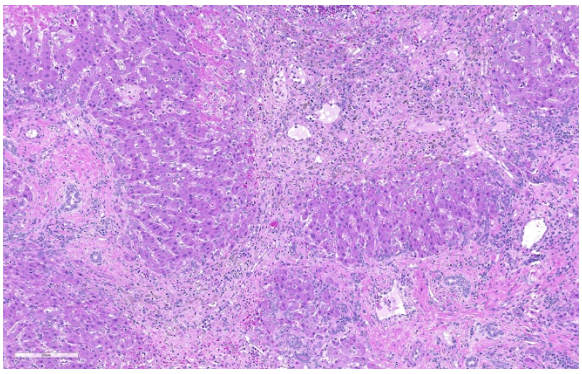
http://www.ufpel.edu.br/fvet/oncovet/
http://www.ufpel.edu.br/fvet/lrd/

**JPC Diagnosis:** Liver: Fibrosis, portal, bridging and random, diffuse, moderate to severe, with arteriolar thrombosis, larval trematodes, and migration tracts
2. Liver, centrilobular hepatocytes: Necrosis, multifocal.

**JPC Comment:** The contributor does an excellent job of describing the pathogenesis and clinical issues surrounding *F. hepatica* in cattle. While most commonly associated with infection in ruminants, *F. hepatica* (and a second related species, *F. gigantea*) can result in fascoliasis in a much wider range of species, including pigs, dogs, cats, capybara, and humans. (WSC 2010-2011, Conference 10 Case 1, details a case of *F. hepatica* in a pony from Ireland.)

*F. hepatica*, termed the temperate fluke, is found on every inhabited continent, while *F. gigantean* is found in tropical areas of Asia and Africa.[3] The life cycle (as described above) is similar for both flukes. Interestingly, in areas of Japan, Vienam, and Korea, a number of intermediate hybrids between the two species have been identified by morphometric and molecular analysis. [3]

Two possible sequelae to hepatic trematodiasis are black disease (*C. novyi*) in sheep and bacillary hemoglobinuria (*C. hemolyticum*) in sheep and cattle. The migration of immature flukes through the hepatic parenchyma may result in the generation of necessary ischemic conditions for the proliferation of *C. novyi* spores, already within the liver, to proliferate. Once active, *C. novyi* produces a necrotizing beta toxin and a hemolytic phospholipase C, resulting in large areas of coagulative necrosis in the liver and death, often with no premonitory signs. The pathogenesis of bacillary hemoglobinuria, caused by *Clostridium haemolyticum*, is similar to black disease with respect to the fluke involved, germination of spores, and toxin production. The toxins

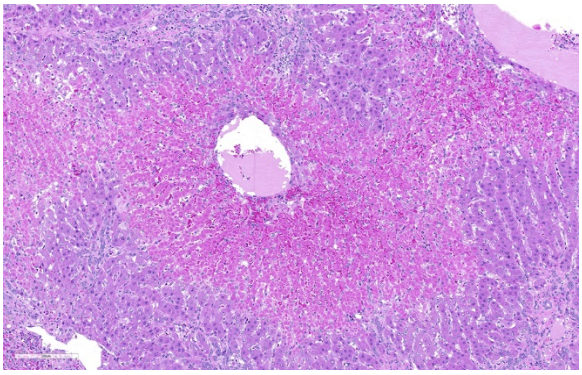


***Liver, ox: There is marked expansion of portal areas with fibrosis and biliary hyperplasia, as well as bridging portal fibrosis. (HE, 137X)***

of *C. haemolyticum* also produces intravascular hemolysis with associated anemia and hemoglobinuria.

Fascioliasis is a tropical disease in humans whose prevalence is likely underestimated given the lack of surveys in endemic areas. Humans may be infected as a result of ingesting metacercariae-infected water or aquatic salad vegetation, such as watercress.[3] Two main phases exist in the human disease. The acute phase of the disease coincides with the migration of immature flukes through liver liver, One to three months following ingestion of the metacercariae, the course of disease is marked by fever, right quadrant pain, hepatomegaly, eosinophilia and hypergammaglobulinemia. The second, or biliary stage, corresponds to active cholangitis with or without cholestasis, andthe presence of flukes within biliary radicles.[1] Diagnosis may be accomplished by stool examination for ova and parasites, but is unreliable during acute stages. The most widely used diagnostic technique is an ELISA test for antibodies against secretory/excretory products from the flukes themselves. Ultrasound or CT results may demonstrate the hyperplastic changes in bile ducts and periductal fibrosis, as well as capsular fibrosis and subcapsular hemorrhage, changes quite similar to that seen in affected animals.[1] While generally easily treated with anti-fluke drugs such as triclabendazole and bithionol, developing resistance to these drugs poses a significant problem for both animals and humans and poses a "One Health" problem for veterinarians and physicians alike.

There is significant slide variation in this case, with not all sections having the arteriolar thrombosis and areas of coagulative centrilobular necrosis. The etiology of the centrilobular necrosis was largely attributed to vascular insufficiency resulting from



***Liver, ox: Multifocally, there are areas of centrilobular and midzonal coagulative necrosis. (HE, 137X)***

arteriolar thrombosis, however the possibility of shock as a cause could not be completed excluded.

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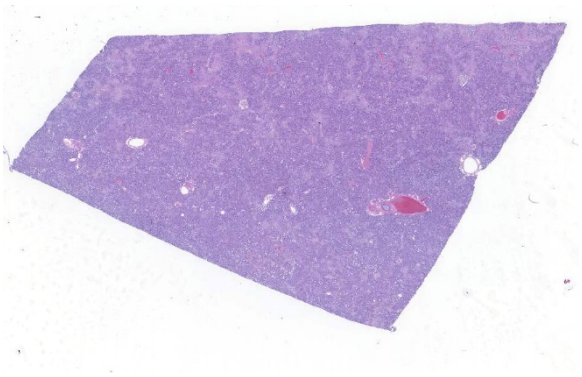
**CASE IV:** UW-SVM Case 2 (JPC 4018823).

**Signalment:** 1-year-old, intact male, Boxer, canine (*Canis lupus familiaris*)

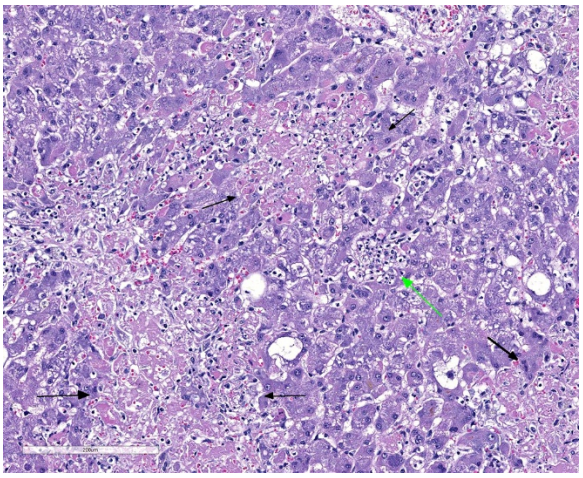
**History:** This dog was under treatment for 4 months for waxing and waning episodes of pyrexia, cervical pain and ataxia (cerebellar and proprioceptive), which progressed to a non-ambulatory state. In addition, valvular endocarditis was diagnosed 2 months following the initial presentation, based on a



***Liver, dog. A retiform pattern of pallor delineates coalescing areas of necrosis throughout the section. (HE, 5X)***

2/6 cardiac murmur and an echocardiogram which showed a vegetative lesion on the aortic valve. Complete blood count showed a neutrophilic leukocytosis. Treatment included non-steroidal anti-inflammatory drugs and various antibiotics (amoxicillin/ clavulanic acid, sulfadimethoxine, cephalexin, ciprofloxacin) for suspected bacterial endocarditis. When negative test results were obtained for a variety of infectious diseases (see laboratory results), a presumptive diagnosis of immune-mediated meningoencephalomyelitis was made and the dog was placed on an immunosuppressive dose of prednisone; antibiotic treatment was continued. There was clinical improvement which then mildly regressed. Azathioprine was added to the immunosuppressive protocol; dramatic clinical improvement ensued which lasted approximately 6 weeks. The dog then showed signs of profound lethargy, weakness, a return of ataxia and mild cervical pain, dyspnea, tachycardia, and pale mucus membranes. The cardiac murmur had resolved. Further testing and treatment were declined and the dog was euthanized.

**Gross Pathology:** The dog was in poor body condition with marked skeletal muscle wasting and scant adipose stores. The liver was diffusely tan, variably friable to mildly firm, and enlarged (4.8% body weight). The lungs were diffusely rubbery, wet, and heavy,



***Liver, dog. Areas of both coagulative (black arrows) and lytic (green arrow) necrosis are present within the section. (HE, 176X)***

and oozed abundant serosanguinous fluid on section; the parenchyma contained four poorly demarcated tan to grey semi-firm nodules ranging from 0.7 cm to 2.5 cm in widest dimension. The trachea contained pink foam and dark red, cloudy watery fluid from the larynx to the carina. The sternal and tracheobronchial lymph nodes were moderately enlarged. The pancreas was pink mottled tan and adjacent fat and omental fat had multifocal firm yellow foci (pancreatitis with fat necrosis). Adrenal cortices were bilaterally thin. There were few small raised crusted foci on metatarsal and metacarpal skin. Cardiac valves were grossly normal, indicating resolution of previous valvular endocarditis.

**Laboratory results:**

**Antemortem results:**

Complete blood count: Neutrophilic leukocytosis at initial presentation. Mild anemia and mild neutrophilia 1 week prior to euthanasia. Blood culture, urine culture: no growth. Cerebrospinal fluid (CSF) analysis: increased protein, neutrophilic pleocytosis, no organisms seen. Blastomyces EIA negative. *Neospora caninum* IFA negative. Rocky Mountain Spotted. Fever titer negative. Lyme C6 4DX SNAP: negative for *Ehrlichia canis, Dirofilaria immitis, Borrelia burgdorferi, Anaplasma phagocytophilum*.

**Postmortem results:**

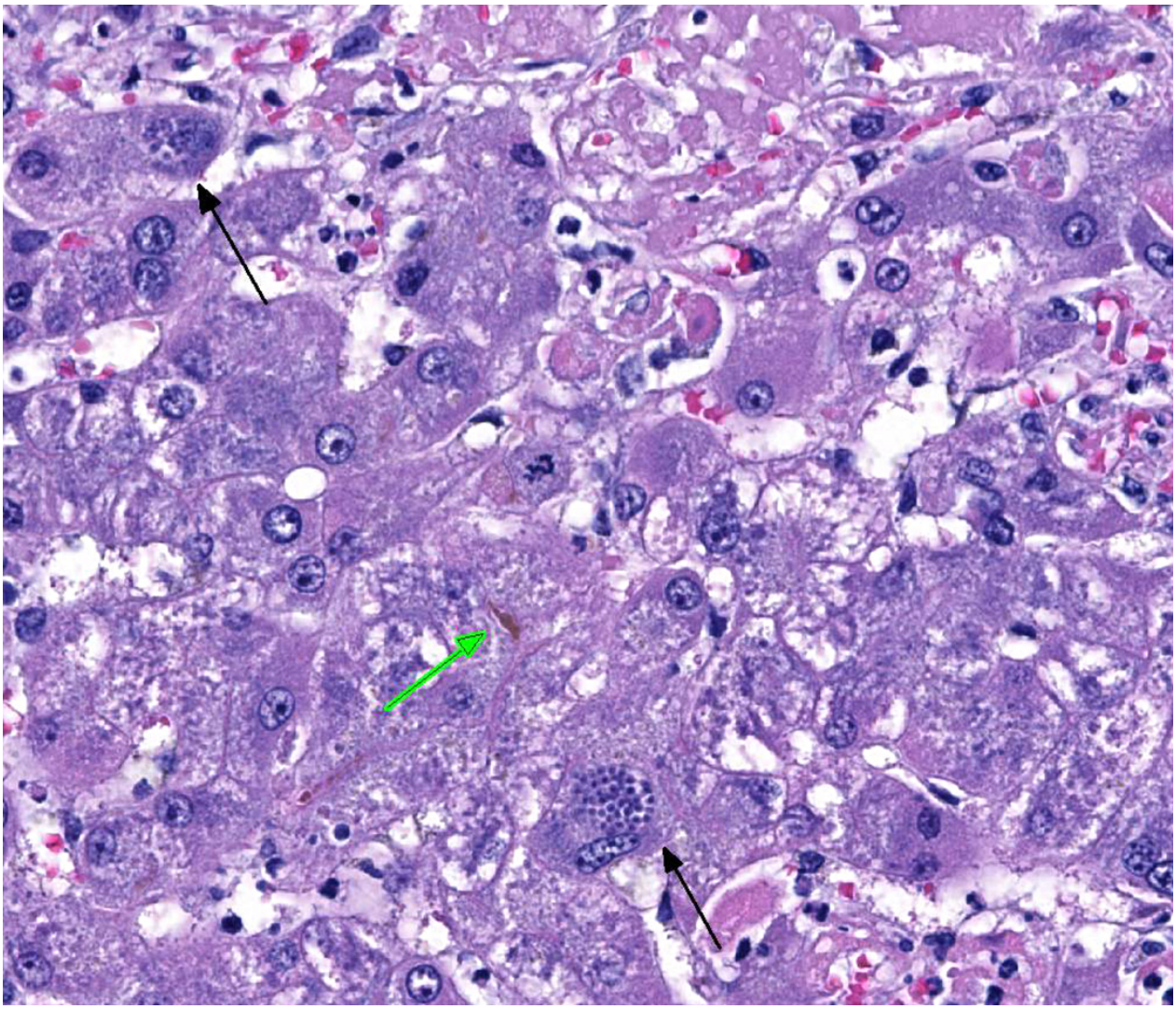
Cytology on lung nodules, impression smear: The sample contained many oval to crescentic protozoal organisms, approximately 2x6 microns, with a 1-2 micron diameter basophilic nucleus, along with many macrophages, fewer degenerate neutrophils, and a heavy background of erythrocytes.

CSF cytology: The sample contained many neutrophils and macrophages; no organisms were seen.

PCR on lung tissue for *Toxoplasma gondii*: positive (Ct=16.92). *Neospora caninum* PCR negative.

**Microscopic Description:**

Liver: Multifocally throughout the liver are many random, occasionally coalescing foci of hepatocellular necrosis, in total affecting up to ~1/3-1/2 of the parenchyma. Hepatocellular necrosis is characterized by one or more of the following: pale eosinophilic cytoplasm with loss of cellular detail and retention of cellular outlines (coagulation necrosis), fragmentation of cytoplasm, karyolysis or karyorrhexis, and replacement of cells by cellular and karyorrhectic debris, fibrin, and few to moderate numbers of neutrophils with fewer macrophages and erythrocytes. Within few hepatocytes, Kupffer cells, and macrophages are small clusters or variable numbers of individual 2-4 micron, round to oval protozoal organisms with a small round basophilic nucleus. Most remaining hepatocytes are mildly to moderately swollen and contain irregular, variably sized cytoplasmic clearings (glycogen-type



***Liver, dog. Numerous zoites bounded by a visible parasitophorous vacuole are present within hepatocytes (and potentially a Kupffer cell at lower right). Bile canaliculi are expanded (cholestasis – green arrow) (HE 400X)***

vacuolar change), with few small random foci and individual cells showing severe swelling and vacuolar degeneration. Multifocally few bile canaliculi are dilated by green-orange bile. Few portal areas are mildly expanded by immature fibrous connective tissue which occasionally extends along surrounding sinusoids; these areas are often bordered by a focus of necrosis.

Additional histologic findings: Multiple other organs also had necrosis with protozoa, including the lung, pancreas, and myocardium, and accompanied by varying amounts of inflammation. The central nervous system had minimal to mild multifocal non-suppurative meningo-encephalomyelitis with microglial nodules and few protozoal cysts that were usually not associated with areas of inflammation. Skeletal muscle had mild myonecrosis with protozoal cysts in few viable myocytes. A focal necrosuppurative skin lesion on the metatarsus contained angioinvasive pigmented fungal hyphae (phaeohyphomycosis). There was bilateral adrenocortical atrophy.

**Contributor's Morphologic Diagnoses:** Liver: 1. Multifocal to coalescing random

hepatocellular necrosis with protozoal organisms

Moderate to marked diffuse hepatocellular vacuolar degeneration, glycogen-type (steroid-induced hepatopathy)

Mild multifocal portal fibrosis; mild extracellular cholestasis

**Contributor's Comment:** *Toxoplasma gondii* is a coccidian protozoal parasite found worldwide that can cause systemic infection in the definitive hosts (members of the Felidae) as well as intermediate hosts (other warm-blooded animals, including humans), though subclinical infections are more common.[3,6]

The life cycle includes an asexual and sexual enteroepithelial cycle that occurs only in felids, and a tissue cycle that occurs in felids and intermediate hosts.[3,6] The host becomes infected by ingesting the organism in the form of sporulated oocysts in cat feces or in material contaminated by cat feces, or in the form of tachyzoites or encysted bradyzoites in tissues of intermediate hosts.[3,4] Vertical transmission can also occur. Tachyzoites replicate in a wide variety of host cells, and spread throughout the body from the intestine via lymphatics or the portal system, within leukocytes or free in plasma.[3] Intracellular replication of tachyzoites causes focal necrosis, which may be followed by inflammation. Tissue cysts containing bradyzoites tend to form in brain and skeletal muscle; immunocompromise may cause these latent tissue cysts to release the zoites and reinitiate systemic infection.[3]

Systemic infection occurs most often in young animals and immunocompromised animals, and in dogs can accompany diseases such as canine distemper, lymphoma, or ehrlichiosis.[3] It has also been reported in dogs undergoing immunosuppressive therapy.[3,6]

Lesions of toxoplasmosis are typically necrosis followed by inflammation, and organs most often affected include the lungs and central nervous system; neurologic and respiratory signs are most common and can be confounding in cases of dual infection with canine distemper.[3] Pulmonary lesions include necrosis of the alveolar septa and interstitial pneumonia. Central nervous system lesions include multifocal necrosis and non-suppurative inflammation, with microglial nodules forming in more chronic cases, but as rapidly as 1-2 weeks following infection. Cysts can form in chronic cases as the infection becomes quiescent.

Hepatocellular glycogen-type vacuolar degeneration (hepatic glycogenosis, glucocortocoid hepatopathy) is due to excess exogenous or endogenous corticosteroids.[5] In this case, an immunosuppressive dose of prednisone caused steroid-induced hepatopathy as well as adrenocortical atrophy. Focal necrotizing dermatitis due to phaeohyphomycosis was presumably an opportunistic infection in this immunocompromised dog.

**Contributing Institution:** Department of Pathobiological Sciences

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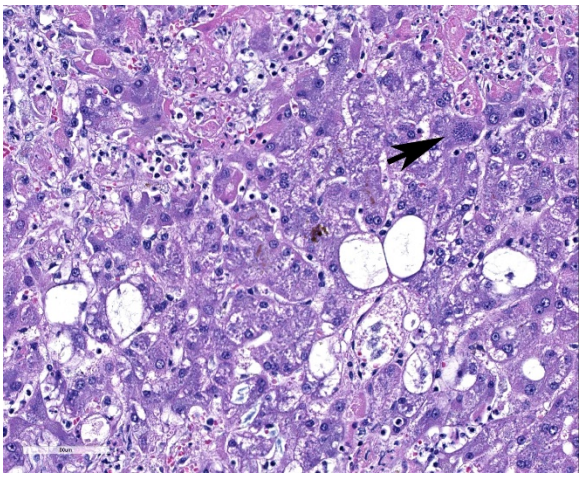
http://www.vetmed.wisc.edu

**JPC Diagnosis:** 1. Liver: Hepatitis, necrotizing, random, multifocal to coalescing, moderate, with edema and intrahepatocytic, intrahistiocytic, and extracellular apicomplexan zoites. 2. Liver, hepatocytes: Glycogenosis, multifocal, moderate.

**JPC Comment:** The contributor has presented an excellent review of the systemic infection associated with *T. gondii*. Within the 15 years, much has been learned about the *T. gondii* infection at the cellular level as well



***Liver, dog. Small groups of hepatocytes are markedly swollen by cytoplasmic glycogen granules ("spider cells"). An intracytoplasmic cyst containing numerous tachyzoites is present (black arrow) and bile canaliculi at center are distended (cholestasis)***

including mechanisms of invasion, replication, and egress, as well as the body's mechanisms to control *T. gondii* and mechanisms that it has evolved to escape those mechanisms.[2]

Interestingly, the parasite forms found intracellularly and extracellularly represent distinct biological states. Extracellular parasites are motile, non-replicative, and designed to extrude the conoid and secrete the contents of their microneme organelles. Intracellular parasites divide, are non-motile and do not secrete micronemes or extrude their conoid. Marked changes in gene expression, mRNA production and translation, and changes in glycolytic pathways of energy production are required from the transition between the two stages.[2]

IFNγ appears to be the lynchpin of mammalian resistance to *T. gondii* infection.[2] IFNγ upregulates a variety of anti-parasite genes whose expression kills the parasite by a number of mechanisms. These include autophagy-dependent degradation of the parasitophorous vacuolar membrane by the p47 family of IFNγ-regulated GTPases (IRGs) as well as the degradation of essential nutrients such as tryptophan by indoleamine dioxygenase. *Toxoplasma* has developed two primary ways of evading these IFNγ-mediated effects. *Toxoplasma* has evolved the ability to produce and secrete proteins which directly inactivate IFNγ-stimulated mediators, including the aforementioned GTPases (effective in most species except higher primates, which do not apparently produce them. Moreover, it can also directly interfere with IFNγ-regulated gene expression, by blocking the binding of STAT1 transcription factors from binding to their target genes.[2]

A number of other cases of toxoplasmosis have been included in the WSC within the last ten years: dog (Liver; WSC 2014 Conf 10 Case 1), cat (Duodenum, WSC 2009-2010, Conference 16 Case 2), and mole (Liver, WSC 2016 Conf 11, Case 4).

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