The Armed Forces Institute of Pathology
Department of Veterinary Pathology

WEDNESDAY SLIDE CONFERENCE 2007-2008

Conference 14

16 January 2008

Moderator:

Keith Steele, DVM, DACVP, PhD

CASE I – 03B 5415 (AFIP 3026837).

Signalment: 6-month-old, intact male, Dalmation, Canine

History: Chronic intermittent vomiting with a recent history of hematemesis and melena. Patchy alopecia on face, left elbow, and right foot.

Gross Pathology: Exploratory surgery revealed multiple acquired extrahepatic shunts. The liver had a greenish tint and accentuated lobular pattern.

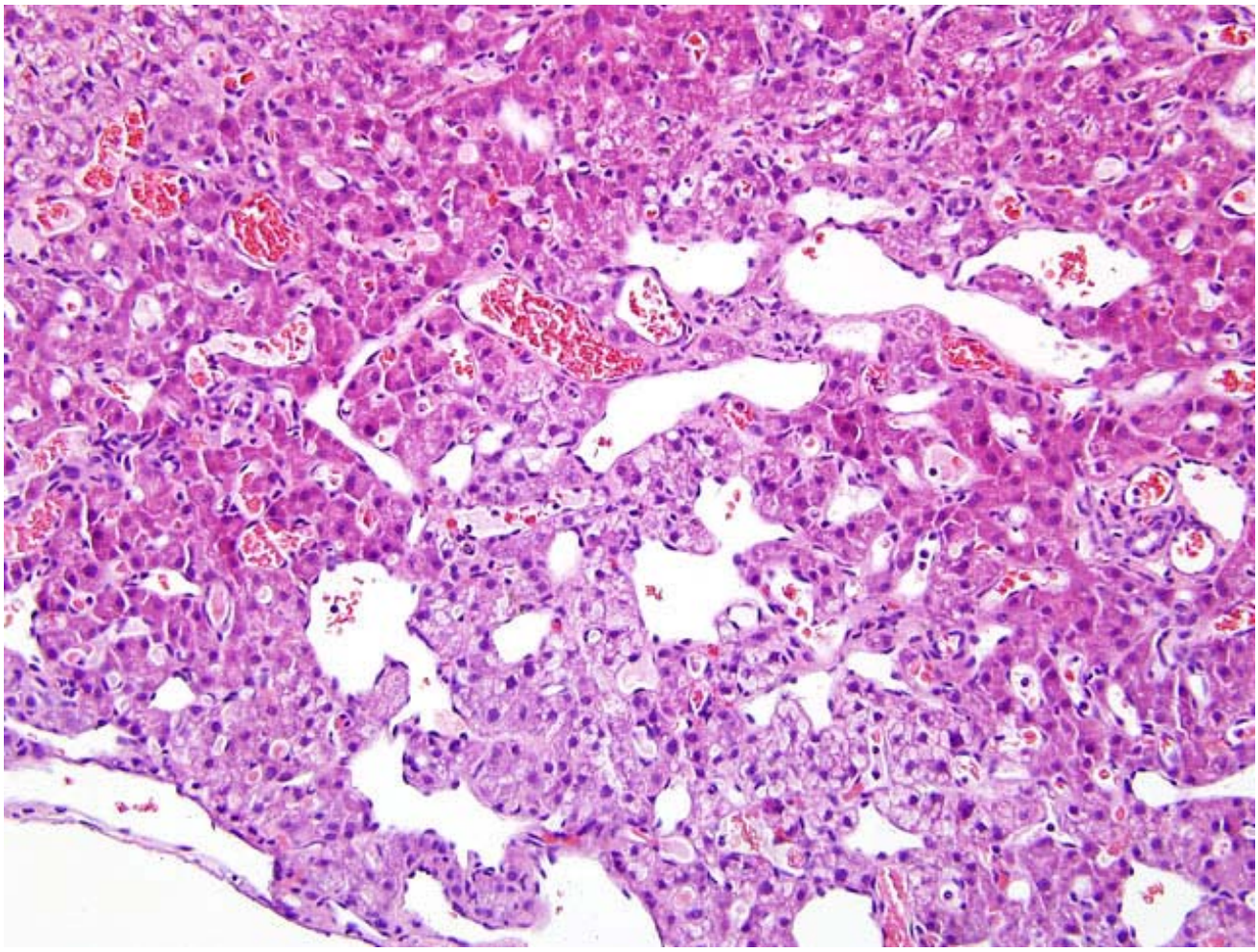
Laboratory Results:
Patient values are followed by reference interval. Anemia: Erythrocytes [3.15 (5.4-8.4)], Hemoglobin [6.9 (12-18)], Hematocrit [20.3 (35-54)], mild neutrophilia and monocytosis - increased ALP [620 (0-100)], ALT [172 (0-60)], AST [100 (0-50)], total bilirubin [0.7 (0.0-0.4)] and cholesterol [364(150-240)], prolonged PTT [24.4 (9.0-12.0)]. Hyperechoic enlarged liver and enlarged gall bladder on ultrasound.

Hepatic copper levels were 459 ppm.

Histopathologic Description: Widespread ectasia and reduplication of portal and central veins is prominent. The venous tunics are thickened by a combination of smooth muscle and fibrous connective tissue. Prominent vascularization and occasional arteriolization of hepatic sinusoids is apparent **(fig. 1-1).** Scattered mixed inflammatory cellular infiltrates are seen in portal and central areas. Centrilobular hepatocytes exhibit variable cell swelling and degeneration. Increased apoptotic bodies and hepatocellular pigmentation is also seen. There is extensive distension of subcapsular lymphatics and/or veins.

Contributor's Morphologic Diagnosis: Liver, severe microvascular dysplasia

Contributor's Comment: Hepatic microvascular dysplasia (HMD) is a syndrome of young to middle aged dogs, which present with signs of liver failure. The most common signs are CNS signs, vomiting, and/or diarrhea. The major differential diagnosis, both clinically and histopathologically, in the younger dogs is portosystemic shunts. In this case, the diagnosis of HMD was made primarily on the basis of the prominent vascularization of the hepatic sinusoids. Additionally, the dilation of the portal veins and minimal duplication of portal arterioles favors HMD over portosystemic shunt. The dilation of portal veins is presumed (though not proven in the literature reviewed by the submitter) to arise from portal hypertension, which could cause the secondary development of extrahepatic shunts as seen in this case.



1-1. Liver, Dalmatian. Sinusoidal ectasia with prominent vascularization (capillarization). (H&E 200X)

Yorkshire terriers and Cairn terriers have higher incidence of HMD, but it has been reported in numerous breeds. There are two hypotheses proposed for the cause of HMD. First is that the persistence of embryonic vitelline veins causes intrahepatic micro-shunts from the portal veins to the central veins. The other is that ultrastructural defects in the sinusoids cause reduced sinusoidal permeability and limited access of plasma components to the hepatocellular surfaces. The prognosis for uncomplicated HMD is better than that of portosystemic shunts. Many respond to dietary management alone and may survive for more than 5 years in good to excellent clinical condition.

AFIP Diagnosis: 1. Liver: Venous dilation, portal and central, diffuse, with lymphangiectasia, mild arteriolar and biliary reduplication, multifocal dissecting fibrosis, sinusoidal ectasia and capillarization, lobule atrophy, multifocal centrilobular hepatocellular degeneration and necrosis, and lipogranulomas, Dalmation (*Canis familiaris*), canine.
2. Liver: Hepatitis, neutrophilic, multifocal, mild.

Conference Comment: The case presented in conference is not typical of microvascular dysplasia or portal vein hypoplasia. Based on the degree of venous dilation and lymphangiectasia and the hepatocellular atrophy, abnormal circulation and portal hypertension are suspected. The process appears centered on the sinusoids with sinu-

soidal capillarization and possibly expanded basement membranes beneath them. Given the loss of lobular architecture, scattered mild fibrosis, mild inflammation, individual cell necrosis and pigment accumulation, this may be an example of lobular dissecting hepatitis in resolution with secondary portal hypertension. Lobular dissecting hepatitis, a form of cirrhosis of unknown etiology reported in young dogs, is characterized by dissection of the lobular architecture by fibroblasts and thin strands of extracellular matrix into small groups of hepatocytes, with accompanying mild to moderate inflammation and hepatocellular apoptosis or necrosis.[2]

Hepatic microvascular dysplasia is a poorly characterized condition with often confusing or contradictory descriptions in the literature on the disease etiology, description, and pathogenesis. The most current characterization, provided by the World Small Animal Veterinary Association (WSAVA) Working Group on Liver Disease and published in 2006, describes the condition as being no different from primary portal vein hypoplasia. The group prefers the latter term as more descriptive of the disease process.[2] Histologically, portal vein hypoplasia shares many features with congenital portosystemic shunts, intrahepatic arteriportal fistulas, and portal vein obstruction, including absent or diminished portal vein profiles and increased numbers of arteriolar profiles.[2] This standard was published after the submission of this case as a Wednesday Slide Conference submission, so this classification was not available for inclusion in the contributor's comments.

We thank Dr. John Cullen, Dr. Yvonne Schulman, and Dr. Thomas Lipscomb for their review and consultation of this case.

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CASE II – CSU 067-69403 (AFIP 3065440).

Signalment: Eleven-month-old, female, intact, bobcat (*Lynx rufus*)

History: In summer, 2006, the kitten was found orphaned at approximately 2 months of age and taken to a wildlife rehabilitation facility. In September, the animal developed slowly progressive neurological disease including a head tremor, nystagmus, ataxia and loss of hind limb function. The animal was considered unsuitable for reintroduction, humanely euthanized and submitted to the Colorado State University veterinary diagnostic lab by the Colorado Division of Wildlife.

Gross Pathologic Findings: The animal was in moderate body condition with minimal autolysis. The brain had been removed immediately following euthanasia; both fresh and fixed brain was submitted with the carcass. No abnormalities were observed on gross post mortem examination.

Laboratory Results:
1. Rabies (FA, brain): Negative
2. Canine distemper virus (PCR, brain): Negative
3. *Toxoplasma gondii* (PCR, brain): Negative
4. Canine parvovirus/feline panleukopenia virus (PCR, brain): Positive
5. West Nile virus (IHC, brain and spinal cord): Positive
6. West Nile virus (PCR, brain): Negative

Histopathologic Description: Present on each slide are sections of spinal cord taken from the cervical and mid-thoracic region. Blood vessels in both grey and white matter are cuffed by lymphocytes, plasma cells and rare macrophages that often extend into adjacent neuropil. In rare sections perivascular hemorrhage is present. Glial nodules are present in both the grey and white matter. Most commonly in areas of gliosis, individual neuronal cell bodies are degenerate or necrotic characterized by hypereosinophilia, loss of Nissl substance, nuclear

pyknosis and variable neuronophagia. Occasionally perikaryonic vacuolization and rarefaction of the neuropil is present. Astrocytes numbers are moderately increased and often surrounded degenerate neurons (satellitosis). Within the white matter there is a variable amount of axonal degeneration, spongy change and occasional digestion chambers. The severity of lesion varies between submitted sections.

Contributor's Morphologic Diagnosis: Spinal cord; lymphoplasmacytic myelitis, chronic, moderate with neuronal necrosis, astrocytosis, perivascular cuffing and glial nodules

Contributor's Comment: West Nile virus emerged as a significant pathogen of birds, humans and horses in the northeastern United States in 1999. The arthropod-borne *Flavivirus* subsequently expanded north and westward resulting in widespread morbidity, and variable mortality, in susceptible species. Reports of WNV infection in non-avian wildlife are largely opportunistic and to our knowledge infection has not been previously reported in a bobcat.

Histologic lesions observed in this case are consistent with those previously reported in other mammals including horses,[1] fox squirrels,[2] white-tailed deer[4] and a dog[5]. The discordance between the WNV PCR and IHC results in this case may reflect the protracted nature of the disease and available tissue for testing. In the brain only very rare neurons stained weakly with IHC while neuronal cell bodies and occasional leukocytes in the spinal cord had abundant antigen; however only fresh brain, and no spinal cord, was available for PCR. The paucity of staining in the brain may represent remnant antigen while no RNA was present for amplification.

Classical gross and histological evidence of CPV or FPV infection in brain or gastrointestinal tract were absent in this case. The PCR product was sequenced and determined to be canine parvovirus 2b; the significance of this finding is unknown. Parvoviral infections have been reported in numerous wild carnivoirs[8] and it has been suggested that CPV 2a and 2b are more common in large, wild cats compared to domestic felids.[7] Recently, parvovirus infection has been reported in association with non-suppurative meningoencephalitis in dogs and cats and proposed as a new parvoviral disease pattern;[6] similar lesions were observed in the brain of this bobcat. Canine parvovirus is also widely distributed throughout the environment and the positive PCR result may be the result of contamination of the tissue sample during brain removal.

AFIP Diagnosis: Spinal cord, cervical and thoracic segments (per contributor): Myelitis, lymphoplasmacytic, multifocal, mild, with moderate axonal degeneration, bobcat (*Lynx rufus*), feline.

Conference Comment: Following its initial identification in the United States in 1999, West Nile Virus has subsequently spread throughout most of the United States and the southern parts of Canada. The virus is genetically divided into two lineages.[3] Lineage 1, occasionally highly virulent (clade 1a), is seen in North America and other areas of the world.[3] Lineage 2, usually non-pathogenic or only mildly virulent, is present primarily within enzootic areas of Africa.[3]

The virus is maintained in the environment within the wild bird population through a bird-mosquito-bird cycle. *Culex* spp. are the primary vectors of transmission, although the virus has been identified in ticks. Additionally, transmission has been documented through direct contact and via fomites.[3]

Histologic lesions often can be very mild even in severe disease and include nonsuppurative encephalomyelitis, gliosis, and glial nodule formation with occasional neuronal degeneration and necrosis.[3] The primary target cell is the neuron with additional damage to microglial cells.[9] Apoptotic cell death appears to be the mechanism of neuronal injury.[9] Conference participants' slides were quite variable in the presence and severity of perivascular cuffing and hemorrhage.

Primarily an infection of birds, WNV has also been documented in horses, humans, ruminants, cervids, canids, felids, squirrels, rodents, and swine.[2,3,4]

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CASE III – TAMU-1 2005 (AFIP 2984049).

Signalment: Two-year-old, male, Weimaraner, *Canis familiaris*

History: The patient had a chronic vomiting problem of one-year duration. The dog was thin with atrophy of all muscles except those of the neck and tongue. Radiographs showed a hiatal hernia. The tongue was difficult to exteriorize for anesthesia due to a large sublingual "mass". Front limbs became spastic during anesthesia. Electromyogram demonstrated spontaneous activity, complex repetitive activity, and high frequency discharges, but motor nerve conduction velocities were normal. Myoglobinuria was noted. Due to the clinical diagnosis, this dog was euthanized.

Gross Pathology: The dog had left side abdominal cryptorchidism and right side renal agenesis. A left side esophageal hiatal hernia contained the stomach and duodenum. Most importantly, the dog had massive thickening of the muscles of the base of the tongue, and that musculature pulled the mandible caudally. The tongue was short and triangular with a base 10cm in diameter (the "mass" noted clinically). The neck muscles were thick, giving a "buffalo hump" appearance. The diaphragmatic muscle surrounding the central tendon was pale and 1.5cm thick; however, overall short. The body muscle mass was reduced and muscles were pale.

Laboratory Results: Serum Creatinine Kinase 32672 u/L (reference range – 68 – 400 u/L); Serum Alanine Aminotransferase 305 u/L (reference range 10 –130 u/L); WBC 21,500 cells/u/L (reference range – 6,000 – 17,000) with an absolute neutrophilia of 18,050 cells / u/L.

Histopathologic Description: The slide presented is of the diaphragm of the patient and a normal size and age-matched dog. On subgross, one notes the obvious and impressive difference in thickness of the longitudinal sections. The thickness is attributed to fibrosis, degenerating hypercontracted, hyalinized, broken and thick fibers with central fiber cysts and nuclei within fibers, as well as on-going regeneration and hypertrophy with proliferation of satellite muscle. The "resident" fat of the diaphragm remains. Mineralization is present.

Contributor's Morphologic Diagnoses: Diaphragm – Severe, diffuse, myodegeneration and necrosis with mineralization and fibrosis and on-going myoregeneration (muscular dystrophy).

Contributor's Comment: The lesions are typical of the muscular dystrophy described in Golden Retrievers.[6,7,8] Immunostaining for dystrophin showed absence of dystrophin (a membrane-associated protein) below the membranes of muscle fibers from the sublingual area, sternohyoideous, and infraspinatus. Thus, this case represents another breed with Duchenne-like muscular dystrophy. Similar X-linked muscular dystrophy has been demonstrated in Golden Retrievers, Labrador Retrievers, Irish Terriers, Samoyeds, Rottweilers and the Japanese Spitz.[1,5] Affected animals lack the subsarcolerminal protein, dystrophin. Clinically, they show progressive weakness and later cardiac abnormalities. This dog also had a dilated and hypertrophic myocardium with severe cardiomyopathy. The unusual presenting clinical complaint, chronic vomiting, is presumed due to the hiatal hernia. Interestingly, Duchenne-like muscular dystrophy researchers using Golden Retrievers found a left side hiatal hernia in their breeding colony.[9] Deficiency of the 427 KD dystrophin protein has been demonstrated in humans, cats, dogs and mice.[2,3,4,8]

The obvious difference in thickness of the longitudinal sections is attributed to hypercontracted, hyalinized, broken, swollen fibers, some having central cysts and central nuclei. These fibers are often separated by extensive fibrosis. Some fiber hypertrophy with sarcolemmal nuclei proliferation is ongoing.

3-1. Diaphragm, Weimaraner. Myofibril size variation with occasional large rounded myofibers. Skeletal muscle hypertrophy. (H&E 200X)
3-2. Diaphragm, Weimaraner. Skeletal muscle necrosis characterized by loss of cross striations, hypercontraction and fragmentation of cytoplasm. (H&E 200X)
3-3. Diaphragm, Weimaraner. Skeletal muscle regeneration characterized by myofibers with a small diameter, slightly basophilic cytoplasm and internal rows of large euchromatic nuclei. (H&E 200X)

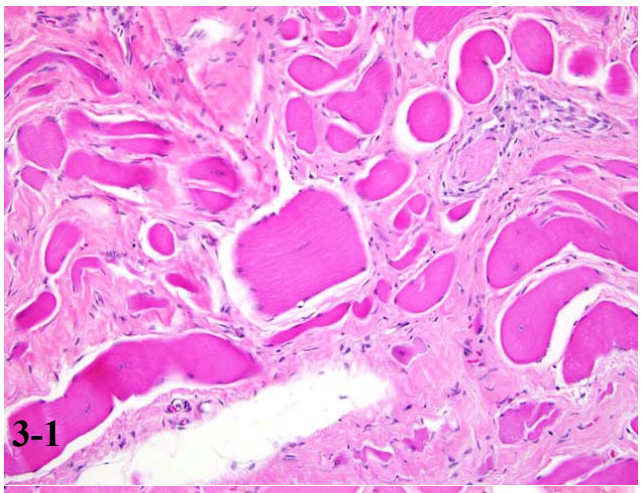
AFIP Diagnosis: Skeletal muscle: Myocyte hypertrophy, degeneration, necrosis, regeneration, and mineralization, diffuse, severe, with fibrosis, Weimaraner, (*Canis familiaris*), canine **(fig. 3-1, 3-2, 3-3).**

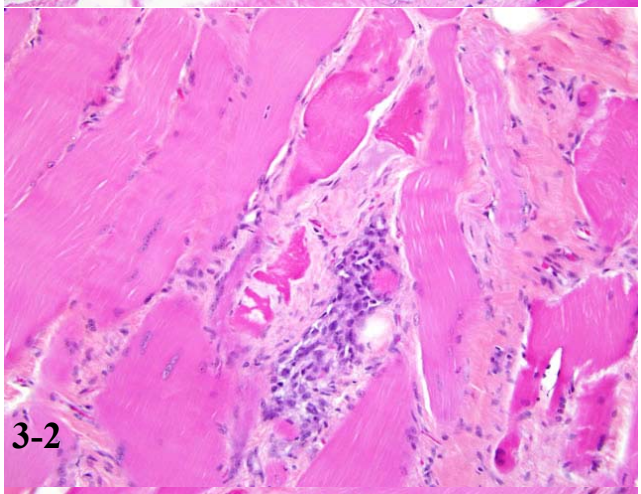
Conference Comment: X-linked muscular dystrophy, an X-linked recessive defect in the dystrophin gene, affects approximately 50% of males born to female carriers.[10] The dystrophin gene codes for a membrane-associated cytoskeletal protein that is present in skeletal and cardiac muscle. The lack of this gene increases the susceptibility of the muscle fibers to repeated bouts of necrosis, regeneration, and fibrosis.[10] Dystrophin deficiency generally results in progressive muscle atrophy of most breeds of dogs, but may cause marked muscle hypertrophy in cats, mice, and Rat Terrier dogs.[3,11]

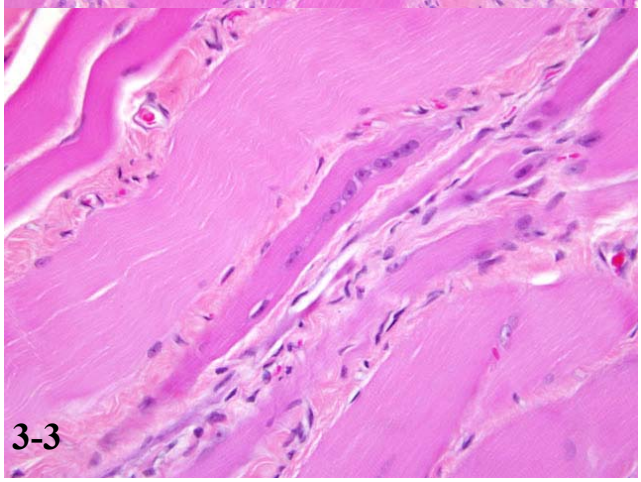
Characteristic gross pathological findings include severe degeneration of the diaphragm and strap muscles with pale white streaks within the affected muscles.[11]

Not all canine muscular dystrophies are X-linked. A defect in sarcoglycan, a component of the sarcolemmal dystrophin glycoprotein complex, occurs in both male and female dogs.[10]

Negative immunohistochemistry for the dystrophin protein is helpful in diagnosing muscular dystrophy, although a positive result will not rule out the entity. Partial expression of dystrophin may occur in Becker-type mutations or in revertant fibers, in which genetic mutation allows some dystrophin expression.[11]







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CASE IV - 2006 AFIP #1 (AFIP 3031544).

Signalment: 7-month-old, female, neutered, German Shepherd Dog (*Canis familiaris*).

History: This dog was singly kenneled in a training center which had 60 other dogs. This dog was found weak and ataxic, with vomiting and diarrhea and was euthanized. It was reported to be eating and drinking and to have had normal feces the day before. Ovariohysterectomy had been performed on this animal 10 days prior to euthanasia. A transitory diarrhea that resolved with oral metronidazole was present for a few days after surgery but no other complications were reported. All other dogs at the facility remained clinically normal.

Gross Pathologic Findings: Approximately 150 ml of sero-sanguineous fluid and large fibrinous clots were present in the thoracic cavity. There were numerous fibrinous adhesions between the visceral and parietal pleura and petechial hemorrhages were present in the intercostal muscles. The ventral 50-80% of all lung lobes was firm, black and depressed. Randomly scattered within these dark areas, were numerous white to pale pink, 1-4mm diameter irregular foci. The pericardial sac was thickened and edematous, with fibrinous adhesions to the visceral pleura.

Laboratory Results: A pure culture of hemolytic *Escherichia coli* was isolated from the lung. Serotyping classified the isolate as O4:H5 or O4:H56 and it tested positive for cytotoxic necrotizing factor 1 (CNF1). Samples of lung were negative for canine influenza by reverse-transcription PCR (RT-PCR).

Histopathologic Description: Most alveoli and bronchioles contain extravasated erythrocytes, eosinophilic proteinaceous fluid, fibrin and an inflammatory exudate of viable and necrotic neutrophils, with fewer macrophages and lymphocytes. There is extensive coagulative septal necrosis, with foci of complete parenchymal dissolution and replacement by necrotic cellular debris. In some sections focally extensive hemorrhage disrupts the normal architecture of the lung. Blood vessel necrosis and fibrin thrombosis are prominent in these areas. Colonies of short rod-shaped bacteria are present in many bronchioles and scattered throughout the necrotic parenchyma **(fig. 4-1).** There are scattered clumps of amorphous, basophilic material (consistent with mineral) in alveolar spaces. There are extensive subpleural hemorrhages.

In the tracheobronchial lymph nodes there was lymphocellular necrosis, hemorrhage and medullary hemosiderosis. Widespread, acute centrilobular hepatic necrosis was the only other significant finding.

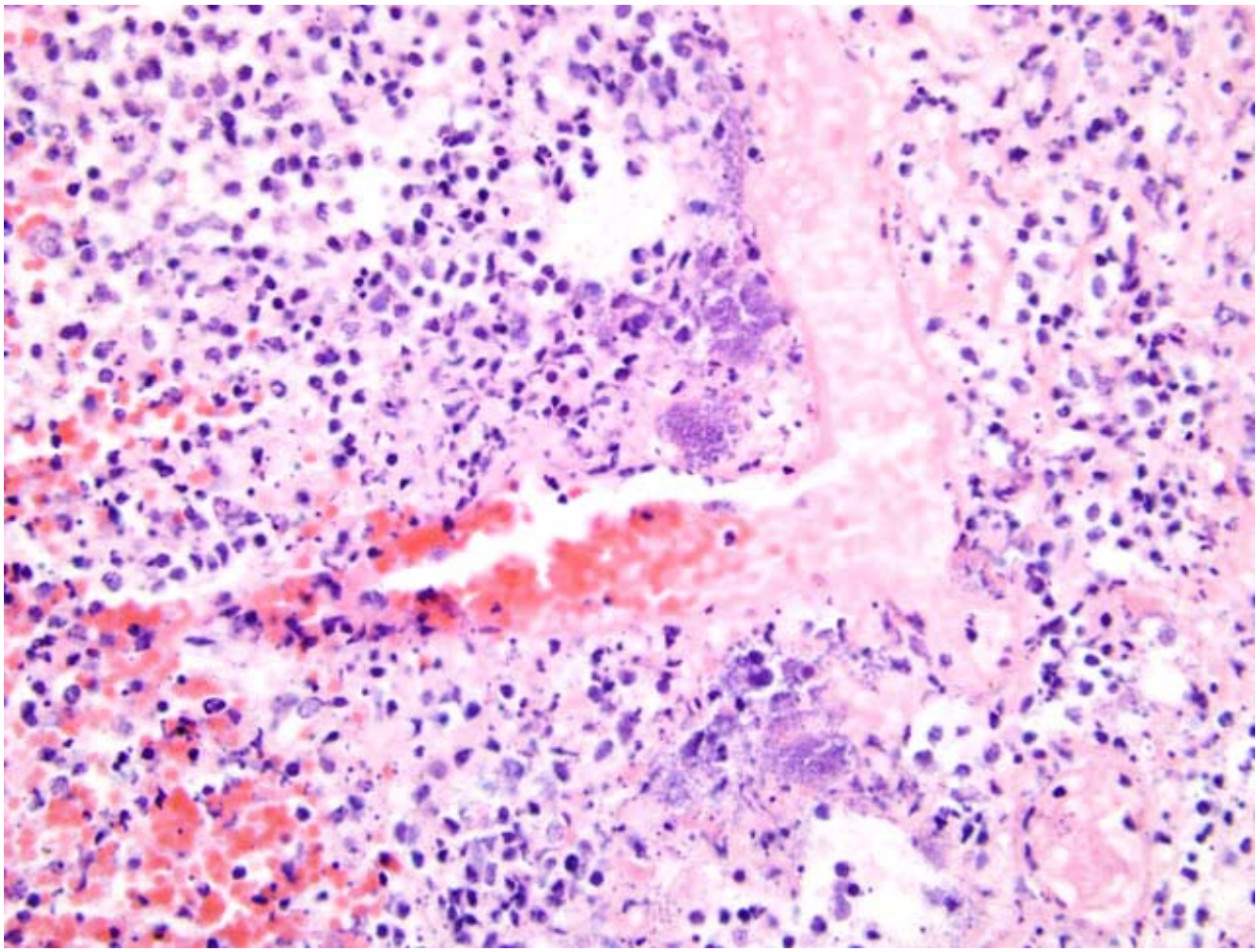
Contributor's Morphologic Diagnosis: Lung: Pneumonia, necrotizing and fibrinohemorrhagic, acute, diffuse, severe with thrombosis and rod-shaped bacteria, etiology consistent with necrotoxigenic *Escherichia coli*.

Contributor's Comment: *Escherichia coli* is the predominant facultatively anaerobic enteric bacterium of

most vertebrates and is frequently isolated from diagnostic specimens. Most strains are either commensals or opportunistic pathogens of immunocompromised individuals, but other strains are well recognized pathogens. The pathogenic strains are historically classified into enteropathogenic, enterotoxigenic, enteroinvasive, enterohemorrhagic, necrotoxigenic and enteroaggregative strains according to the O (lipopolysaccharide) and H (flagellum) serotype, and are further characterized by their production of virulence factors.[5] Cytotoxic necrotizing factor 1 (CNF1) is consistently produced by necrotoxigenic *E. coli* and some isolates also produce CNF2 and alpha hemolysin. Necrotoxigenic strains are an important cause of extraintestinal disease such as urinary tract infections, pyometra, meningitis, septicemia and pneumonia in humans and mammals.[5]

Hemorrhagic and necrotizing pneumonia caused by necrotoxigenic *E. coli* has recently been described in dogs.[1,4] In common with the present case, affected dogs have been young (<1year), the clinical illness is usually less than 24 hours and immunodeficiency or concurrent illness were not identified. In our case, parainfluenza virus and adenovirus testing were not performed and while no inclusion bodies were identified, concomitant infection with these agents cannot be excluded. *E. coli* of both O4 and O6 serotypes have been reported to cause these lesions, which irrespective of serotype were positive for CNF1.

The main differential diagnoses for hemorrhagic pneumonia in dogs are canine influenza and bacterial septicemias including streptococcal septicemia.[2,3] Microscopically, canine influenza is characterized by a pneumonia that is more broncho-interstitial and suppurative with less necrosis[2], but RT-PCR or virus isolation is best per-



4-1. Lung, German Shepherd. Colonies of rod-shaped (bacilli) admixed with inflammatory cell infiltrates and cellular debris in a necrotic focus. (H&E 200X)

formed for definitive exclusion. Samples of lung from this case were negative for canine influenza by RT-PCR.

Little is known about the source and route of infection, means of transmission and pathogenesis of this disease in dogs.

AFIP Diagnosis: Lung: Pneumonia, necrohemorrhagic, neutrophilic and histiocytic, diffuse, severe, with fibrin, edema, and numerous bacilli, German Shepherd Dog (*Canis familiaris*), canine.

Conference Comment: Strains of *E. coli* are identified by the various antigens they express, primarily using the O and H antigens.

- O antigens (somatic): Determines the serogroup, lipopolysaccharide molecule
- H antigens (flagellar): Determines the serotype
- K antigens (capsular): Made up of polysaccharides and proteins; may also be used for classification purposes
- Fimbrial or pili antigens: Important in adhesion and colonization of epithelium

Extraintestinal pathogenic *E. coli* have been associated with pyometra, mastitis, otitis, prostatitis, bacteremia, skin diseases, cholecystitis, and pneumonia. Strains producing the cytotoxic necrotizing factor (CNF) are referred to as necrotoxic *E. coli*.[4] These strains produce either CNF1, identified in humans and domestic animals, or CNF2, identified only in ruminants.[4,5] The genes that code for CNF-1 and alpha hemolysin are genetically linked and have a tendency to occur with O4 and O6 groups.[1,4]

The primary fimbrial antigen in extraintestinal pathogenic *E. coli* is the P fimbriae and is encoded by the *pap* (pilus-associated pyelonephritis) gene.[1,4] The *papG* (fimbrial tip adhesion) and *papA* (major fimbrial subunit) alleles have also been associated with necrotoxic *E. coli*.[1,4]

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Notes: