The Armed Forces Institute of Pathology
Department of Veterinary Pathology

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Conference Moderator:

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CASE I – UFSM-2 (AFIP 3105599)

Signalment: 2-year-old, male, mixed breed, bovine (*Bos taurus*)

History: This bovine belonged to a herd of 2-year-old cattle that were placed in a pasture highly infested with bracken fern (*Pteridium aquilinum*). Five cattle (including the one of this report) died after presenting clinical signs that included high fever, hemorrhages through the nose, petechiae in mucous membrane and hematuria. The 12 cattle had been placed in the bracken fern-infested pasture in mid-October (spring) and the deaths occurred from late December to early January (summer). When examined for the first time, the bovine of this report was depressed and had a rough hair coat, few petechiae in the ocular mucosae, slightly red discolored urine, soft stools with small blood clots and rectal temperature of 40.2°C. In the following days the rectal temperature peaked to 42.2°C and the clinical signs got worse with frank hematuria, hemorrhages through the nose, depression, and foul smelling black soft stools with blood clots. This bovine died after a 6-day clinical course (the other 4 affected cattle died after clinical courses that varied from 25 days). In the terminal phase of the disease the rectal temperature dropped to 39°C.

Gross Pathology: Large hemorrhages were seen in the subcutaneous tissue of the trunk and abdominal cavity, mainly in the areas of attrition. Multiple 0.5 cm diameter subepicardial hemorrhagic foci were observed in both ventricles. The natural and cut surfaces of lymph nodes and spleen were hemorrhagic. Petechiae, ecchymosis and hematomas were observed in the omentum and mesentery **(Fig. 1-1)**, in the intestine at its junction with the mesentery and in the serosa of the fore stomachs. Several small ulcers were observed in the abomasal mucosa and a large (17x4 cm) cylindrical blood clot was observed in the lumen of the abomasum, near the pylorus. There were ulcers covered with blood in the intestinal mucosa mainly over the Peyer's patches. Partial blood was admixed with the contents of the large intestine and a large cylindrical clot (15x3 cm) was observed in the lumen of the cecum. The mucosa of the ureters were markedly edematous and hemorrhagic. The bladder contained approximately 200 ml of urine and blood, and the bladder mucosa was swollen and hemorrhagic. There was partially clotted blood in the joint cavities of the pelvic limbs.

Laboratory Results: Severe thrombocytopenia and neutropenia were seen. Below are results of blood tests performed on the day before the bovine of this report died.

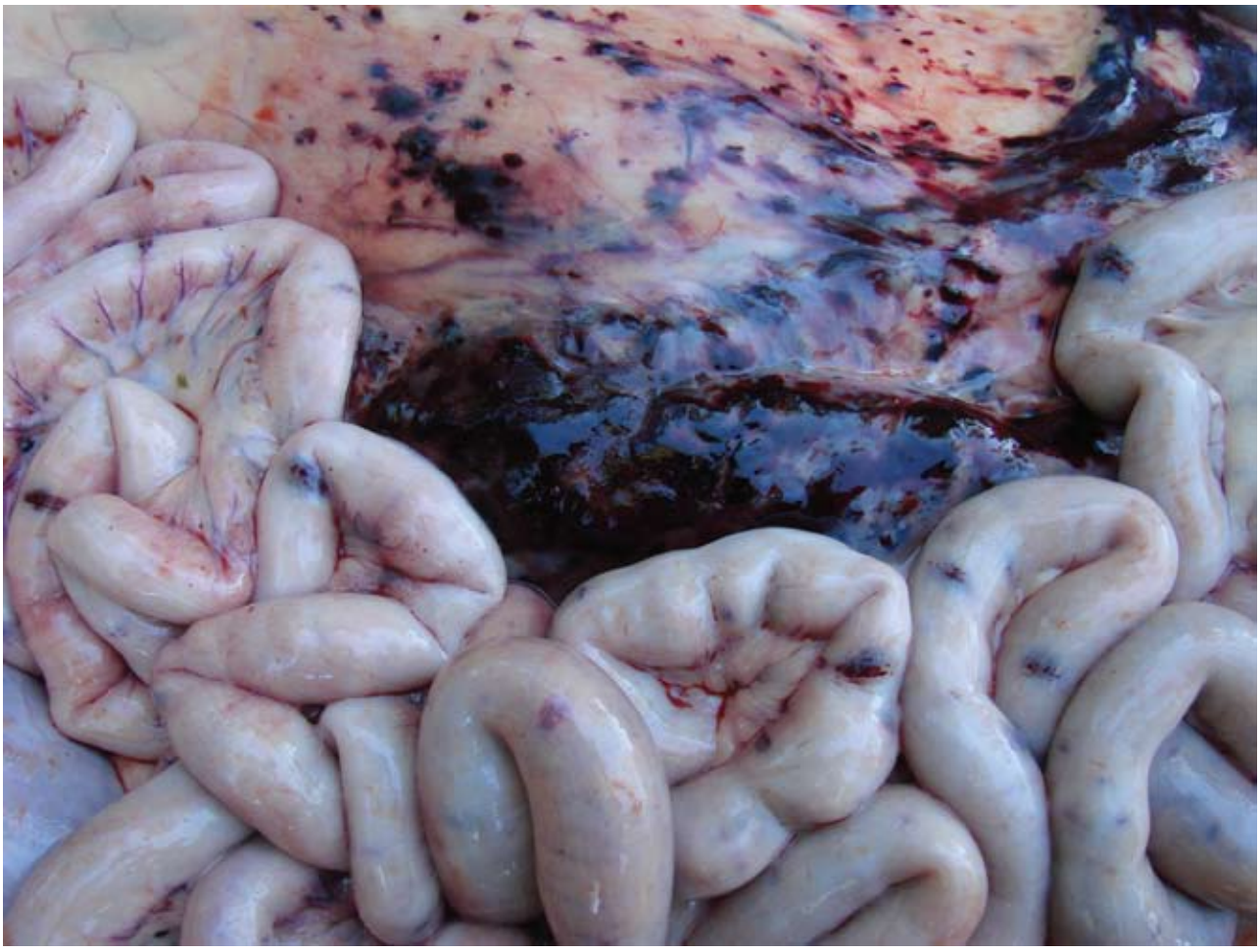
*Sponsored by the American Veterinary Medical Association, the American College of Veterinary Pathologists, and the C. L. Davis Foundation.

Blood coagulation tests

Parameter	Unity	Reference values	Affected bovine
Partial time of activated thromboplastin	seg	25-45	49.3
Prothrombin time	seg		20.2

Complete blood cell count

Parameter	Unity	Reference values	Affected bovine
Red blood cells	($x10^6/mm^3$)	(5.0-10.0)	5.1
Hemoglobin	(g/dl)	(8.0-15.0)	7,9
Packed cell volume	(%)	(24-46)	23
Mean corpuscular volume	(fl)	(40.0-60.0)	45.1
Mean corpuscular hemoglobin concentration	(%)	(30.-36.0)	34.3
Leucocytes	($/mm^3$)	(4,000-12,000)	5,100
Neutrophils	(%)	(15%-45%)	0
	($/mm^3$)	(600-4,000)	0
Lymphocytes	(%)	(45%-75%)	100
	($/mm^3$)	(2,500-7,500)	5,100
Monocytes	(%)	(2%-7%)	0
	($/mm^3$)	(25-840)	0
Eosinophils	(%)	(0%-20%)	0
	($/mm^3$)	(0-2,400)	0
Basophils	(%)	(0%-2%)	0
	($/mm^3$)	(0-200)	0
Platlets	($x10^3/mm^3$)	(100-800)	3

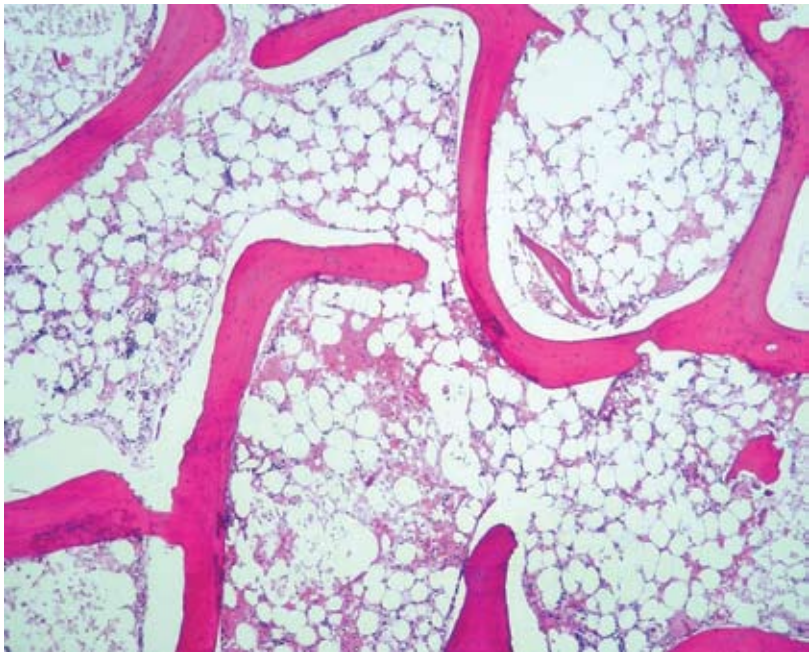


1–1 Omentum, mesentery, and small intestine, ox. Multifocal to coalescing petechial and ecchymotic hemorrhage. Photograph courtesy of Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv

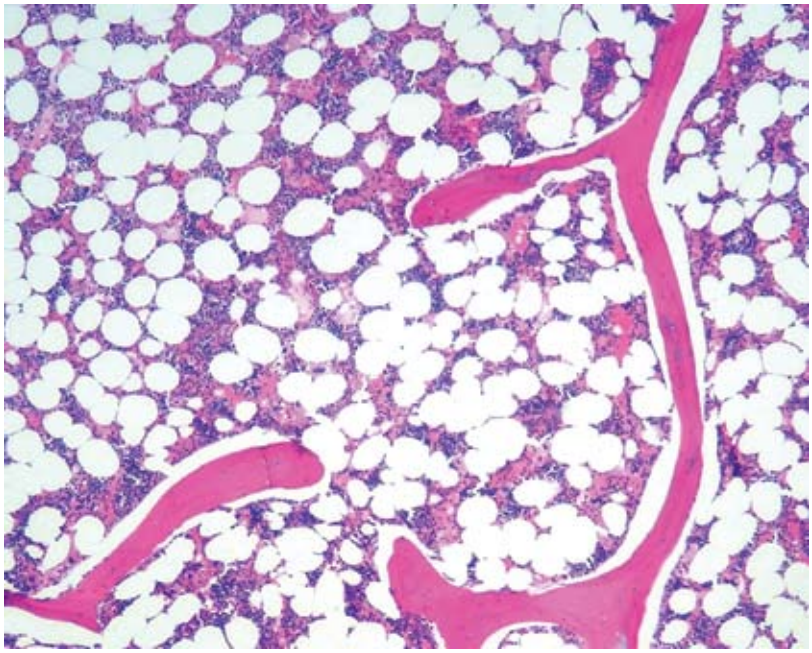
Histopathologic Description: The hemorrhages observed grossly were confirmed on histopathological examination. Sections of the bone marrow of the sternum were prepared from the affected bovine **(Fig. 1-2)** and from a normal age matched control **(Fig. 1-3)**. Both sections are arranged in the same glass histoslide submitted. There was severe aplasia of the bone marrow of the affected bovine as compared to the control. The sinusoids of the marrow are distended due to the lack of pressure usually posed by the large numbers of hematopetic cells. There is paucity or absence of nucleated cells, including megacariocytes, which can be observed in normal regular numbers in the control section.

Contributor's Morphologic Diagnosis: Bovine, bone marrow, sternum, aplasia, severe,
Etiologic diagnosis: toxic bone marrow aplasia
Etiology: toxic substance(s) in *Pteridium aquilinum*
Condition: acute poisoning by *Pteridium aquilinum*

Contributor's Comment: Bracken fern (*Pteridium aquilinum*) is the second most important plant poisoning in southern Brazil and responsible for 12% of all cattle deaths caused by poisonous plants in this region.[11] The ingestion of bracken fern results in three forms of clinical disease in cattle (two of those are chronic and characterized by development of neoplasms): 1) squamous cell carcinomas in the upper digestive tract (base of tongue, esophagous and entrance of the rumen) [13]; 2) several types of the neoplasms in the urinary bladder.[14] This latter condition is associated with bleeding from the urinary bladder and universally known as enzootic hematuria. The cocarcinogenesis of bracken fern with bovine papillomavirus-4 (BPV-4) and BPV-2 is implicated respectively in the pathogenesis of upper digestive tract squamous cell carcinomas and bladder tumors. [11, 12] 3) A third condition caused by the ingestion of the plant is characterized by bone marrow aplasia and generalized bleeding tendency [17] and is the one affecting the bovine of this report. This condition



1–2 Bone marrow, ox. Severe bone marrow aplasia. Photomicrograph courtesy of Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv



1–3 Bone marrow, ox. Bone marrow from a normal age-matched control. Photomicrograph courtesy of Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv

is generally referred to as the acute form of bracken fern poisoning. It is mostly a disease of cattle but has been occasionally reported in sheep. Acute bracken fern poisoning in cattle was originally reported in England at the end of 19th century as an acute disease characterized by high fever, hemorrhages and high lethality rates.[16] During this time the etiology was only suspected but it was confirmed in the following year [1] and in later years by successive experiments in cattle being fed with large amounts of the plant for prolonged periods.[4,5,9,15] The same disease was observed during the autumn of 1917-1920 in cattle from the state of New York [2] but only confirmed later as being caused by the ingestion of bracken fern.[5] Since then the disease has been reported from several other countries.[17,18]

Clinical signs associated with acute form of bracken fern poisoning in cattle include high fever (up to 42.5°C), severe

hemorrhages in various tissues and organs, neutropenia and thrombocytopenia.[4,8,10,15,16] High fever appears to be the first clinical sign of the disease, and some believe [14] the intensity of fever that occurs in acute bracken fern poisoning is not duplicated by any other diseases in cattle. Fever is followed by hemorrhage through nasal cavity and depression. In the advanced stages of the disease the fever may subside.[4,9,10,12]

Acute bracken fern poisoning in cattle has been subdivided by some [10, 12] into two types: 1) *enteric*, which is the most common and characterized mainly by depression, anorexia, high fever, and weak pulse, blood clots in the feces and foul smelling feces, pale mucous membranes and bleeding from mucous membranes of the nose, eyes, vagina, and anus; and 2) *laryngeal*—with clinical signs including high fever, roaring, laborious breathing and edema of the larynx. In our experience, however, it is not uncommon to observe signs of both types overlapping in the same animal, as is the case of the bovine of this report. Hemorrhages from venipunctures, from insect bites and in the milk are commonly observed. Death ensues usually 2-3 days after the first clinical signs; however peracute cases of 4-10 hours and cases of recovery are also reported.[4,17] In our region (unpublished data) acute bracken fern poisoning generally occurs in spring and summer months, in hill camps where the ecology is conducive of fern growth, with morbidity rates of 12.5%-21.5% and high lethality rates.

The primary lesion in the acute bracken fern poisoning in cattle is a severe suppression (aplasia) of the bone marrow (as observed in the bovine of this report) which results in thrombocytopenia and neutropenia. No changes are observed in red blood cell [9] and any deficit in RBCs numbers are due more to the hemorrhaging than to the bone marrow suppression since the RBC half-life in cattle is considerably greater (157-162 days) than that of platelets (5-10 days) and neutrophils (12 hours). Usually death due to bleeding related to thrombocytopenia occurs when the numbers of platelets are lower than 10,000/mm^3 of blood. In these cases fatal bleeding within body cavities occurs. In the case of this report platelets were 3,000/mm^3 one day before death.

There is usually bacteremia related to the almost complete lack of neutrophils in the peripheral blood.[4,9] If after the onset of the bacteremia the bovine survives for several days, infarcts may occur in the liver, lung, kidney and spleen. Death occurs because of internal hemorrhage or septicemia.[10,12]

The most remarkable necropsy findings are hemorrhages of almost any size in almost any organ.[10,12,15] Petechiae and ecchymosis occur in the subcutaneous tissue and there is edema and hemorrhages in lymph nodes; petechiae and paint-brush hemorrhages are observed on serosal surfaces of several organs from abdominal and thoracic cavities. Abundant collections of partially clotted blood can be found in the peritoneal cavity. Mucosal ulcerations and blood clots in the lumen of fore stomachs, abomasum, small and gross intestine are observed. Pale necrotic areas of necrosis can usually be observed in the liver but also in the heart, kidney and spleen. These areas are usually referred to as infarcts but probably are caused by toxins of bacteria and not really by ischemia due to compromise of the vasculature. Pharyngeal and laryngeal edema can be marked.[10,12,16]

The hemorrhages observed grossly can be confirmed histologically. Additionally, foci of coagulative necrosis associated with clusters of bacteria can be found in the liver, heart, kidney and spleen. The most striking histological change is the severe aplasia of the bone marrow.[4,9]

The norsesquiterpene glucoside ptaquiloside is the toxin in *P. aquilinum* responsible for the bone marrow suppression in acute disease [6]; this toxin has cumulative properties and the time elapsed from the beginning of the ingestion of the plant and the development of the clinical signs will depend of the amount of ingested plant.[4,12] Usually the ingestion of daily amounts equal to or above than 10 g/kg/bw during 2-11 weeks are necessary to produce the disease.[4,9]

The list of differential diagnosis for acute bracken fern poisoning in cattle should include anaplasmosis, pneumonic mannheimiosis (differential for the laryngeal form), septicemia pasteurellosis, leptospirosis, sweet clover poisoning, bacillary hemoglobinuria.[12] A similar disease caused by bone marrow suppression is reported in cattle from Australia and is caused by the ingestion of an yet different poisonous plant (*Cheilanthes sieberi*).[3]

AFIP Diagnosis: Bone marrow: Hypoplasia, trilineage, diffuse, severe

Conference Comment: Bracken fern toxicosis occurs in several domestic species including pigs, horses, sheep and cattle. Susceptibility depends on the species affected, with cattle and horses being highly susceptible. Sheep rarely eat this plant and are thus affected less often, and pigs are rarely affected.[7,8,19] The plant is most toxic when it is green and actively growing. This plant is not very palatable, so it is often only eaten in large quantities during drought conditions. In monogastrics, bracken fern poisoning leads to a thiamine deficiency. This results in neurologic and cardiac disease most commonly reported in pigs and horses.[7,8,19]

There are numerous toxins within bracken fern including quercetin, ptaquiloside, shikimic acid, aquilide A, and prunasin.[7,8,19] The two major syndromes commonly seen in cattle are aplastic anemia and enzootic hematuria. The severity of disease depends upon how much of the plant is ingested. There is a cumulative affect with this poison. Morbidity is low and mortality is high with this disease. If enough of the toxin is ingested over weeks to months, death via thrombocytopenic hemorrhage or septicemia secondary to neutropenia is often the result.[7,8,19]

In cattle and sheep, bracken fern poisoning results in an irreversible trilineage marrow hypoplasia and resultant aplastic pancytopenia. The chronic form is usually from long term, low level ingestion of bracken fern resulting in persistent hematuria and alimentary tract neoplasia.[7,8,19] Bracken fern toxicity also commonly causes neoplasia within the urinary bladder.[7,8,19] Papillomas, fibromas, and hemagiomas are most common in the urinary bladder.

Hemorrhages can be found in almost any tissue with bracken fern poisoning, but they are usually most numerous in the stomach and small intestine. Multiple neoplasms of the bladder wall or esophagus and forestomachs may also been seen at necropsy. Marrow cellularity is often markedly diminished and the marrow appears pink and soft.[7,8,19]

Contributing Institution: Departamento de Patologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. http://www.ufsm.br/lpv

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CASE II – 1008-1080 (AFIP 3111136)

Signalment: 2-year-old spayed female Boxer Dog (*Canis familiaris*)

History: A 2-year-old female spayed Boxer presented to MSU CVM for chronic anemia. The referring veterinarian administered multiple blood transfusions, and initiated treatment with doxycyline, prednisone and vitamin K. On presentation, the dog was bright and alert. The mucus membranes were pale, and a grade 4/6 systolic heart murmur was auscultated. A complete blood count revealed severe anemia and mild thrombocytopenia. Serum chemistries, PT, PTT, radiographs and abdominal ultrasound were within normal limits. A direct Coombs test was positive. An echocardiogram revealed mild tricuspid regurgitation.

A blood smear was submitted for review.

Gross Pathology: None

Laboratory Results:
CBC -

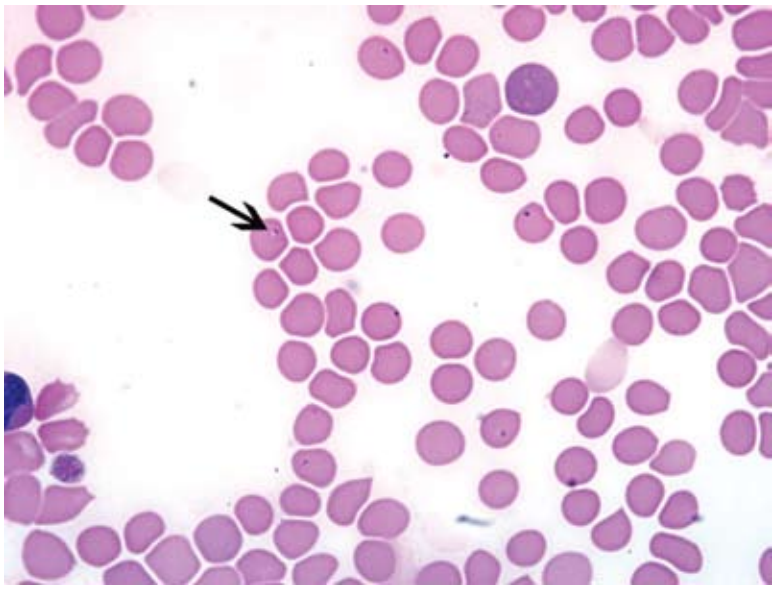
DAY	1	2	3 (post transfusion)
Hematocrit (34-60%)	18.5%	15.8%	30.2%
Platelet estimate	128 K/µL	48 K/µL	128 K/µL
Segmented neutrophils (3,000-11,500/ µL)	12,638/µL	NA	2,686/ µL

Chemistry – no significant abnormalities
Prothrombin time – 7.4 sec (5-12)
Partial Thromboplastin Time 7.8 sec (10-20)
Canine Direct Coombs test – positive
Serology -
Babesia canis – positive 1:320
Babesia gibsoni – positive 1:640
Borrelia burgdorferi – positive 1:640
PCR -
Babesia gibsoni – positive for Asian genotype

Histopathologic Description: A blood smear from a 2-year-old spayed female Boxer Dog is submitted for review. The red cell density is markedly decreased indicating a severe anemia. There is moderate polychromasia and anisocytosis suggestive of a regenerative erythroid response. Scattered Howell-Jolly bodies and metarubricytes are also observed. Small (1-2µm) signet ring-shaped organisms and larger (2-3µm) piriform-shaped organisms are seen individually within erythrocytes **(Fig. 2-1)**. The platelets appear mildly decreased. However, moderate platelet clumping is noted. Many megaplatelets are observed suggestive of a regenerative response to platelet consumption or destruction. The leukocytes appear normal in morphology and a mild left shift is present and is suggestive of an inflammatory process.

Contributor's Morphologic Diagnosis: Severe regenerative anemia; mild thrombocytopenia; intraerythrocytic organisms consistent with *Babesia* species

Contributor's Comment: Canine Babesiosis is a hemoprotozoan infection which is typically caused by *Babesia gibsoni* or *Babesia canis*. *Babesia gibsoni* is small (1 x 3.2µm), pleomorphic, signet ring-shaped, and is usually observed as a single organism within the erythrocyte. *Babesia canis* is larger (2.4 x 5µm), and is generally observed as a paired, pyriform structure within the erythrocyte. Both species have Ixodid tick vectors and are found throughout Africa, Asia, Europe, the Middle East, and North America. *B. canis* is typically more prevalent. However, there has been an increase in the frequency of *B. gibsoni* infections, particularly in North America.[1] The specific vector for *B. gibsoni* in the United States has not been established. *Rhipicephalus sanguineus* and *Dermacentor variabilis* are suspected as possible vectors, but transmission studies have been unsuccessful or inconclusive.



2-1. Peripheral blood smear, dog. Among the erythrocytes, there is mild to moderate anisocytosis and polychromasia. Occasionally, there are 1-2 micron, round to crescent shaped intraerythrocytic merozoites (arrow). (HE 1000X)

The organisms are transmitted through the bite of an infected tick. Once inside the host, the organisms attach to the erythrocyte membrane and are endocytosed. While in the cytoplasm, the organisms undergo binary fission resulting in the formation of merozoites. Ticks become infected during feeding by ingesting merozoites. Transtadial and transovarial (*B. gibsoni*) transmission result in the formation of sporozoites within the tick's salivary glands. While feeding, saliva and sporozoites from the infected tick are passed into the host's circulation. For transmission to occur, the tick must remain attached for 2-3 days.[2]

Infected dogs may present with a variety of clinical signs, ranging from hemolytic anemia to multiple-organ dysfunction syndrome (MODS). Subclinical infection is common in American pit bull terriers, which have a strong breed predilection for *B. gibsoni*. The predominant feature of babesiosis is hemolytic anemia. Thrombocytopenia is also quite common. The anemia is a result of both extra- and intravascular hemolysis. Parasitemia results in increased osmotic fragility, shortened erythrocyte life span, and erythrophagocytosis. The infected erythrocytes have parasite antigens on their surface, which induce opsonization by host antibodies and removal by the mononuclear-phagocyte system. The induction of serum hemolytic factors and production of antierythrocyte membrane antibodies with resulting damage from the secondary immune system also contribute to the pathogenesis of this disease. Other factors thought to be involved include oxidative damage, impaired hemoglobin function, as well as sludging and sequestration of infected erythrocytes.

Diagnosis may be made by identifying the parasite on a blood smear, serology (IFA and ELISA), or PCR which can detect low levels of parasitemia. Coinfection with other tick borne pathogens occurs relatively often as a single vector can harbor several infectious organisms.[1]

AFIP Diagnosis: Cytological specimen, peripheral blood smear: Moderate polychromasia and anisocytosis (regenerative anemia) with intraerythrocytic organisms consistent with Babesia species

Conference Comment: The proposed pathogenesis of babesiosis is as follows: tick transmission →parasitized erythrocytes→hemolysis (intravascular and extravascular) → anemia (hemoglobinemia, bilirubinuria, icterus) →decreased available oxygen for cellular machinery →anaerobic metabolism → acidosis → hypoxic cell damage → shock → death. DIC is often a sequelae of terminal babesiosis.[3]

Differential diagnoses for babesiosis include parasitic, immune-mediated, oxidative, and traumatic causes of hemolytic anemia.[3] Normally, infected animals present with anemia and thrombocytopenia. The anemia starts out as a normocytic, normochromic anemia and progresses to a macrocytic, hypochromic, regenerative anemia. Serum chemistries are generally within normal limits, with hypokalemia sometimes occurring in severely affected animals.[3]

Other intra-erythrocytic parasites of veterinary importance include:

Species affected	Parasite
Dogs	*Babesia canis, Babesia gibsoni*
Cats	*Babesia cati, Babesia felis*
Cattle	*Anaplasma marginale, Anaplasma centrale* *Babesia bovis, Babesia bigemina* *Theileria mutans, Theileria annulata*
Sheep	*Babesia ovis, Babesia motasi*
Horses	*Babesia equi, Babesia caballi*
Deer, Elk	*Theileria cervi*
Birds	*Hemoproteus* spp., *Leukocytozoon* spp., *Plasmodium* spp.

2

Contributing Institution: Mississippi State University College of Veterinary Medicine, www.cvm.msstate.edu

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CASE III – 07H7837A (AFIP 3103707)

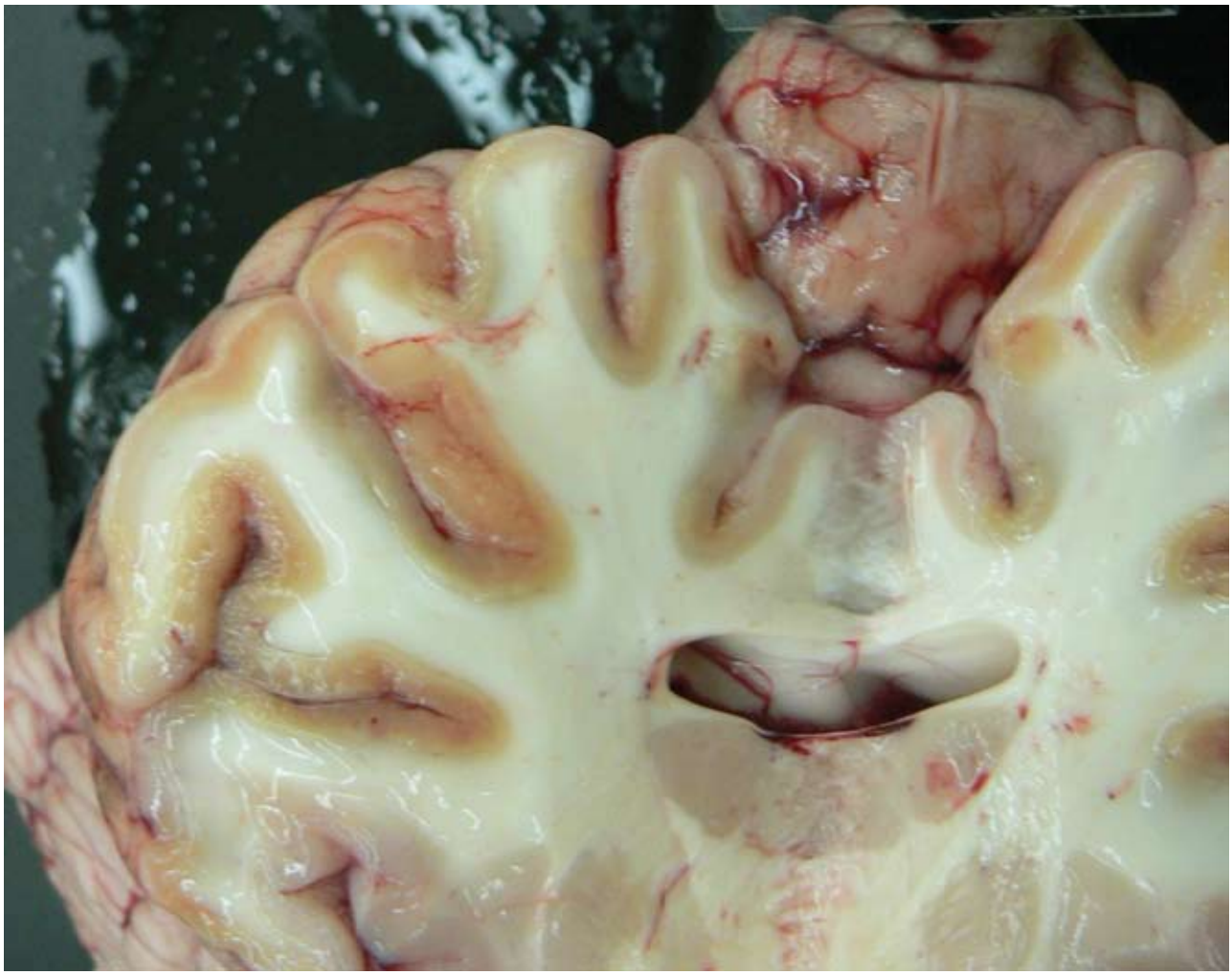
Signalment: 7-month-old Aberdeen Angus bovine steer (*Bos taurus*)

History: The animal presented for a short history of anorexia that progressed to ataxia, head pressing, and ultimately recumbency. Upon presentation, the calf was severely depressed, lethargic, unresponsive and severely ataxic in all four limbs. There was absence of menace reflex bilaterally, and the animal appeared to be blind. The owner stated that the calf was in a group of animals consuming a ration containing corn gluten.

Gross Pathology: Gross lesions were limited to the brain. There were multiple locally extensive areas within the cerebrum where the gray matter was markedly thinned. In these areas, remaining gray matter was characterized by fragmentation, softening and slight yellow to tan discoloration. Multifocally there was cleft formation at the gray matter-white matter junction where gray matter was separated from the underlying white matter **(Figs. 1-1 and 1-2)**. When ultraviolet light was applied to the fresh brain, distinct autofluorescence of these areas was observed **(Fig. 1-3)**.

Laboratory Results: Cerebral spinal fluid: Protein = 578.1 mg/dL; CK = 505 IU/L (normal range 0-5 IU/L); CSF cytology revealed mononuclear pleocytosis; blood lead was negative.

Histopathologic Description: Multifocally the deep cortical gray matter adjacent to underlying white matter is disrupted, fragmented and rarefied in a laminar pattern. There is disruption, vacuolation and/or loss of the neuropil in these areas (malacia). There is loss of cortical neurons, and remaining neurons are often shrunken, angular, hypereosinophilic and contain pyknotic nuclei (neuronal necrosis) **(Fig. 3-4)**. Multifocal remaining neurons are

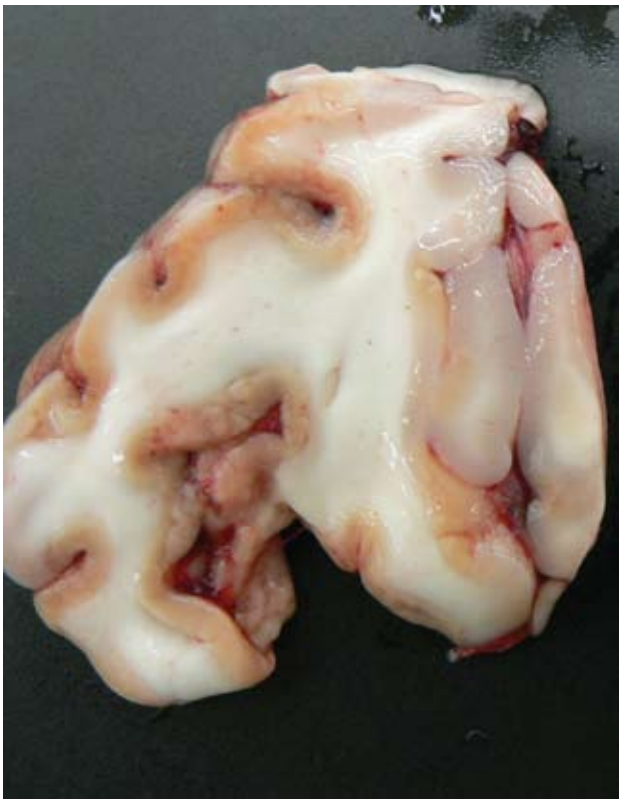


3-1. Cerebrum, ox. Cortical laminar necrosis. Photograph courtesy of Iowa State University College of Veterinary Medicine, Department of Veterinary Pathology; http://www.vetmed.iastate.edu/departments/vetpath

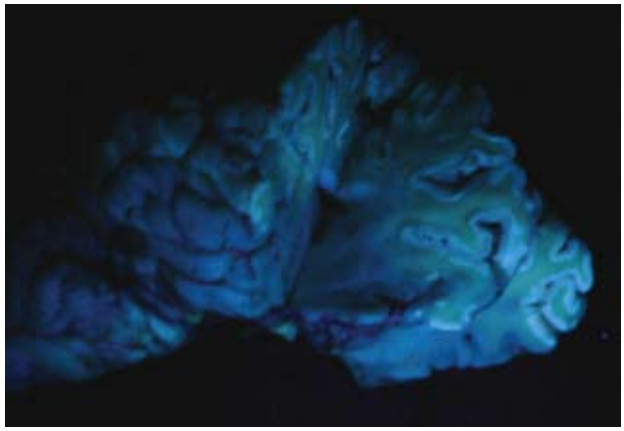
surrounded by glial cells (satellitosis and neuronophagia). Diffusely throughout the sections are increased numbers of glial cells (gliosis) including reactive astrocytes. In addition, there are multifocal random accumulations of erythrocytes (hemorrhage) and moderate numbers of gitter cells with fewer lymphocytes, neutrophils and in some sections karyorrhectic cellular debris. Axonal fibers are occasionally separated and surrounded by clear space (edema) and swollen axonal sheaths contain swollen hypereosinophilic bodies (spheroids) or gitter cells. Vessels are lined by plump endothelial cells (hypertrophy) and are frequently surrounded by clear space (edema) or moderate numbers of macrophages or lymphocytes. In some sections, the meninges are moderately expanded by clear space and low numbers of macrophages.

Contributor's Morphologic Diagnosis: Brain (cerebrum): Necrosis and neuronal loss, cortical, laminar, multifocal, moderate to severe with moderate multifocal histiocytic and lymphocytic meningoencephalitis

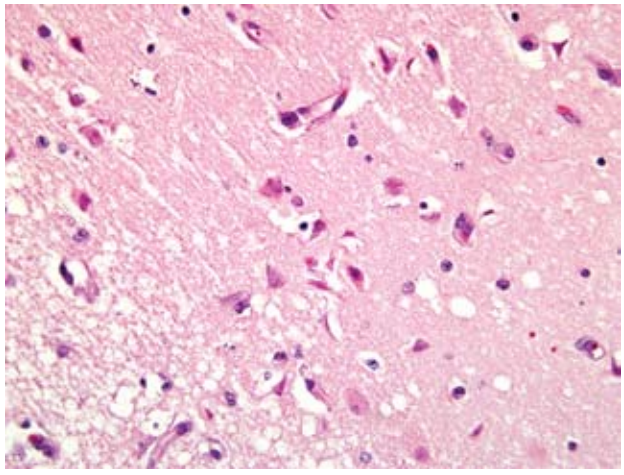
Contributor's Comment: The gross and histologic lesions in the cerebrum of this calf are characteristic of nutritional polioencephalomalacia (PEM) that is commonly seen in growing ruminants. The pathogenesis of nutritional PEM associated with thiamine deficiency is well established in carnivores (progressive encephalopathy); a thiamine-PEM association in ruminants is less clear. PEM in ruminants was thought at one time to be exclusively caused by thiamine deficiency, but it is now known that the laminar cortical necrosis can be caused by sulfur toxicity, lead toxicity, and hypoxia in addition to thiamine deficiency.[2] Confusion regarding the cause of PEM in cattle is partly due to the lack of a method to accurately



3-2. Cerebrum, ox. Cortical laminar necrosis. Multifocally there is clefting and separation of malacic gray matter from the underlying white matter tracts. Photograph courtesy of Iowa State University College of Veterinary Medicine, Department of Veterinary Pathology; http://www.vetmed.iastate.edu/departments/vetpath



3-3. Cerebrum, ox. Cortical laminar necrosis. When ultraviolet light was applied to the fresh brain, distinct autofluorescence of these areas was observed. Photograph courtesy of Iowa State University College of Veterinary Medicine, Department of Veterinary Pathology; http://www.vetmed.iastate.edu/departments/vetpath



3-4. Cerebrum, ox. Within the gray matter, primarily at the interface of white and gray matter, there is rarefaction of the neuropil, multifocal neuronal necrosis, and occasional gemistocytic astrocytes. (HE 400X)

evaluate thiamine status in animals as well as thiamine-responsiveness that is frequently noted clinically.

The basis of sulfur-associated PEM in ruminants appears to be the excessive production of ruminal sulfide caused by the ruminal microbial reduction of ingested sulfur. Soluble hydrosulfide anion is in the rumen fluid phase, and hydrogen sulfide gas accumulates in the rumen gas cap. Non-reduced forms of sulfur (sulfate and elemental sulfur) are relatively nontoxic, while hydrogen sulfide and its ionic forms are highly toxic substances that interfere with cellular energy metabolism.[1] Sulfide inhibits cellular respiration by blocking cytochrome C oxidase in the electron transport chain. It is likely that sulfide produced in the rumen is absorbed into the blood and is capable of inhibiting energy metabolism of neurons and that this either directly causes neuronal necrosis or leads to necrosis by interfering with local cerebral blood flow and creating regional ischemia.[3] The most important route of sulfide absorption is unknown, but potentially occurs via the gastrointestinal mucosa or pulmonary mucosa after inhalation of eructated ruminal gas. The role of thiamine in these cases is not completely clear; however most current evidence indicates that sulfur-associated PEM does not involve reduced concentrations of thiamine in blood/tissues or reduced activity of transketolase, a thiamine-dependent enzyme.[3] A significant portion of PEM cases in ruminants may in fact be sulfur-associated rather than thiamine-associated, as was once thought.

Exposure to large amounts of sulfur can occur from a variety of sources including both feed and water. The

occurrence of PEM in ruminants associated with high dietary sulfur intake has been recognized with increasing frequency, particularly in Midwestern United States. This appears to be associated with the recent expansion of the fuel ethanol industry, as byproducts of corn (maize)-to-fuel ethanol production are being used with increasing frequency in ruminant diets. Ethanol byproducts are attractive feed ingredients due to their nutritional (especially protein) value and they are readily available at reduced cost (compared to corn) in Midwestern states. Sulfuric acid is utilized in ethanol byproduct production, and byproducts thus often contain significantly elevated levels of sulfur which represent a major source of dietary sulfur for these animals. In addition, there is much variation in the sulfur content of ethanol byproducts both within and between plants, and for this reason periodic monitoring is recommended for producers utilizing these products in rations.[2] In this case, the history of corn gluten meal feeding (a commonly fed ethanol byproduct) is considered to be the likely source of elevated sulfur, though feed samples were not evaluated for confirmation. This case represents a classic case of ruminant PEM both clinically and pathologically. Sulfur-related PEM manifests clinically as acute blindness, recumbency, seizures and death or a more subacute form characterized by visual impairment and ataxia. Lesions are classic and are characterized by extensive necrosis of cerebrocortical neurons. Grossly, there is fragmentation, malacia and loss of cortical gray matter that in some cases autofluoresces brightly when exposed to 366-nm UV light. At later stages, affected tissue becomes cavitated as macrophages infiltrate and necrotic tissue is removed.[1]

AFIP Diagnosis: Brain, cerebrum: Necrosis and neuronal loss, cortical, laminar, multifocal, moderate to severe, with edema and moderate multifocal histiocytic and lymphocytic meningoencephalitis

Conference Comment: The contributor does a magnificent job describing PEM in ruminants. Thiamine deficiency in carnivores (Chastek paralysis) is also mentioned and a brief review follows.

Thiamine, or vitamin B1, is an essential dietary need in carnivores. Ruminants are able to produce their own thiamine from the diet via microbial production in the rumen.[3] Thiamine deficiency in carnivores often results from eating a diet high in fish containing thiamine-splitting enzymes (thiaminases). Sulfur dioxide, often used to preserve that "fresh fish" appearance, can destroy thiamine after it is metabolized into sulfates.[3]

If the disease is recognized early in its progression, thiamine supplementation can reverse the course of the disease. If clinical signs are present for several days, and a point of no return is passed, death is the result. Microscopic lesions include vacuolation of the neuropil, vascular dilation, hemorrhage, and necrosis. The periventricular gray matter is highly susceptible and the lesion is almost always bilateral and symmetrical most often involving the inferior colliculi.[3]

Contributing Institution: Iowa State University College of Veterinary Medicine, Department of Veterinary Pathology; http://www.vetmed.iastate.edu/departments/vetpath/

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CASE IV – Case 1 (AFIP 3093118)

Signalment: 8-week-old, intact female, English springer spaniel, (*Canis familiaris*), dog

History: This female English springer spaniel was purchased from a farm. Per clinical history, she showed vomiting and diarrhea followed by icterus and oliguria.

On clinical presentation, the dog had pale and icteric mucous membranes. Her femoral pulses were weak and intermittent with marked deficits. Electrocardiography suggested atrial fibrillation. The dog had tachypnea and increased respiratory effort, with harsh lung sounds on thoracic auscultation.

Acute oliguric renal failure was diagnosed based on clinical signs and laboratory data (see below). Despite

fluid therapy, diuretics and treatment for hyperkalemia, three days following the commencement of clinical signs the dog developed ventricular fibrillation, which progressed to cardiopulmonary arrest that was refractory to resuscitation including defibrillation.

Gross Pathology: The oral mucosa was pale with yellowish discoloration. The subcutaneous adipose connective tissue had yellow discoloration (icterus). The lungs were reddened and firm. The kidneys were pale and bilaterally the cortices contained petechial hemorrhages.

Laboratory Results: A complete blood count revealed minimal neutrophilia 11.7x10^9/L (reference value: 3-11.5 x10^9/L), moderate monocytosis 2.98x10^9/L (reference value: 0.15-1.5 x10^9/L) and a borderline normochronic, normocytic anemia with an HCT of 27.2% (reference value: 31.4%).

Serum biochemistry revealed a moderate panhypoproteinemia 41g/L (reference value: 49-71g/L) due to hypoalbuminemia 21.2g/L (reference value: 28-39g/L) and minimal hypoglobulinemia 19.8g/L (reference value: 21-41 g/L). Further abnormalities included marked hyperkalemia 9.9mmol/L (reference value: 4.1-5.3 mmol/L), marked hypercalcemia 4.12mmol/L (reference value: 2.13-2.7 mmol/L) and marked hyperphosphatemia 6.89mmol/L (reference value: 0.8-2.0 mmol/L). A marked azotemia with elevated blood urea nitrogen (BUN) 64.1mmol/L (reference value: 3-9.1 mmol/l) and elevated creatinine 601µmol/L (reference value 98-163mmol/L) was also present. Other tested parameters included increased amylase 1874U/L (reference value: 176-1245U/L), increased lipase 4470U/L (reference value: 72-1115U/L), hyperbilirubinemia 92.1mmol/L (reference value: 0-2.4 mmol/L) and an increased alkaline phosphatase (ALP) 933 U/L (reference value: 19-285 U/L).

Urinalysis revealed isosthenuria (specific gravity 1.010), pH 7.0 and elevated glucose (3+). Urine culture revealed no bacterial isolates after 48 hours incubation.

Antibody titers to several *Leptospira* spp., which can be pathogenic to dogs, were tested. All Leptospira serovar pool titers were negative.

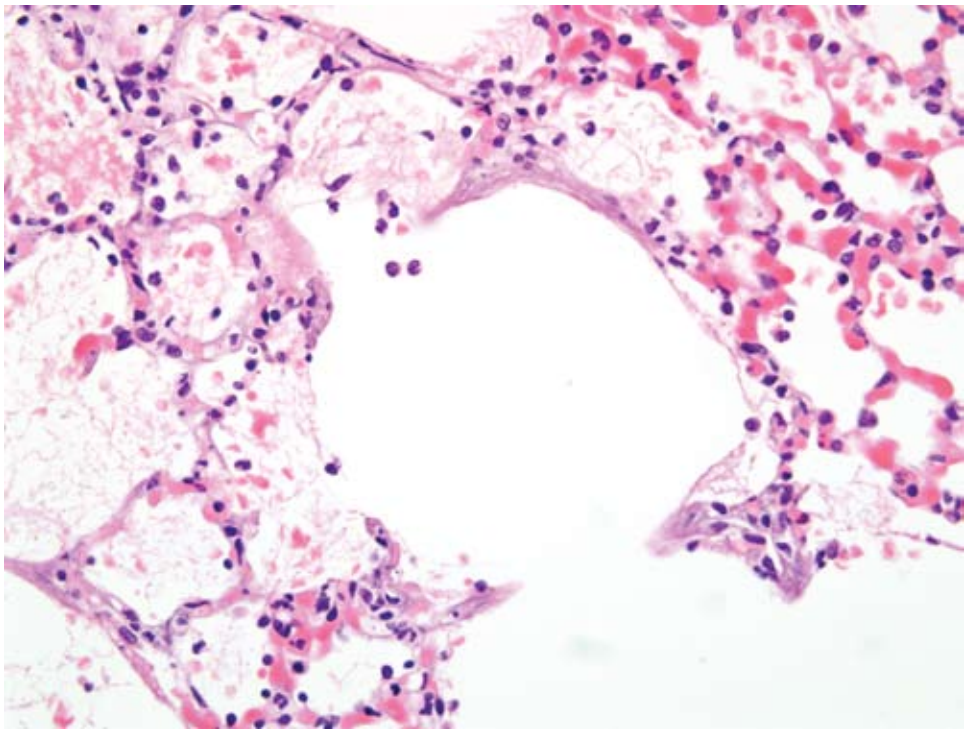
Histopathologic Description: Lungs. There is multifocal deposition of blue granular material within interalveolar septae (interpreted as mineralization, **Fig. 4-1**). Numerous alveolar spaces contain strands of eosinophilic fibrillar material. Multifocally, histiocytes are present in alveolar spaces and often surround mineralized septae. Extravasated erythrocytes and very rare neutrophils are also present in some alveolar spaces. Capillaries and blood vessels are congested and there is moderate to focally marked expansion of the perivascular interstitium by clear spaces (oedema). Bronchiolar lumens contain fibrin strands, small to moderate numbers of erythrocytes and sloughed cellular debris. Brown-black staining of alveolar septae using a von Kossa stain indicates the deposition of calcium salts and thus confirms tissue mineralization **(Fig. 4-2)**.

Contributor's Morphologic Diagnosis: Lungs, alveolar septae; Mineralization with histiocytosis, moderate, diffuse
Lungs, alveoli: Fibrin exudation, moderate with histiocytosis

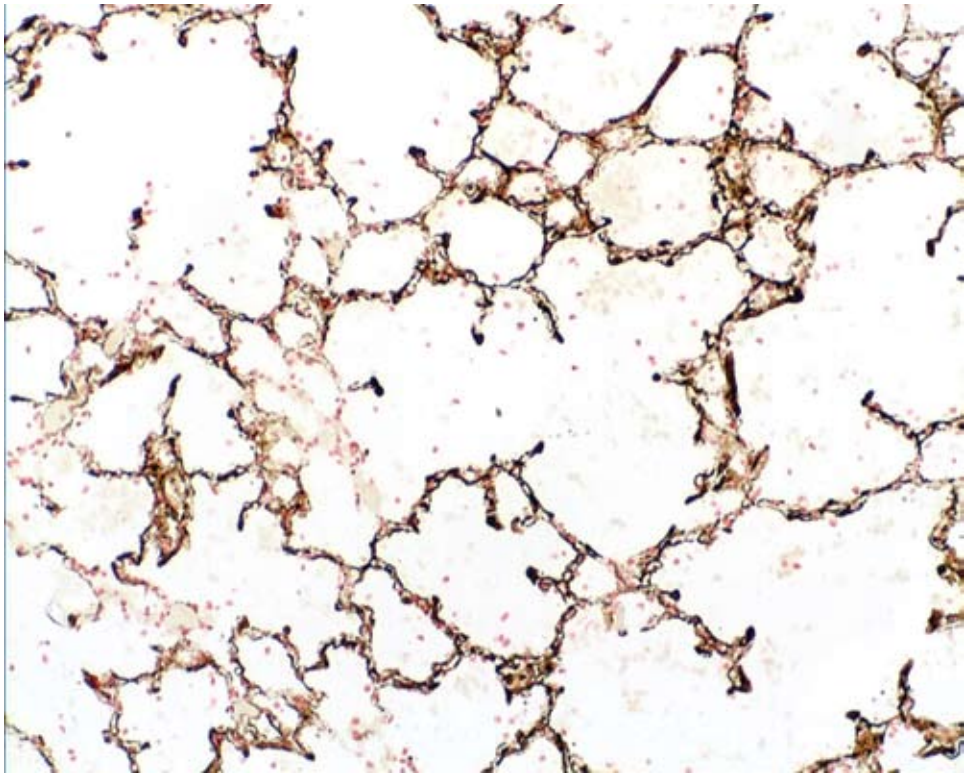
Contributor's Comment: Pathologic mineralization is defined as the abnormal tissue deposition of principally calcium salts, with smaller amounts of other salts, and thus is synonymous with the term tissue calcification.[7] In general, calcification can be dystrophic, occurring locally at sites of tissue necrosis, metastatic, in which calcium deposition occurs in otherwise healthy tissue as a result of altered calcium metabolism or idiopathic if no obvious underlying cause can be detected.[7,11] Metastatic mineralization can be caused by several conditions, which are summarized below.

1) *Hyperparathyroidism*: Hyperparathyroidism is associated with elevated parathyroid hormone/parathormone (PTH) causing bone resorption and thus hypercalcemia.
Primary hyperparathyroidism is characterized by the autonomous secretion of PTH due to parathyroid gland neoplasia.[2,8]
Pseudohyperparathyroidism is caused by the secretion of PTH-like proteins by malignant tumors (hypercalcemia of malignancy) e.g. lymphoma and anal sac apocrine gland adenocarcinoma.[2,8] Non-parathyroid neoplasia has been reported as the most frequent cause of hypercalcemia in dogs and cats.[4]

2) *Bone destruction:* Hypercalcemia can be observed with osteolytic lesions caused by multiple myeloma and tumor metastasis to bone.[5]
3) Vitamin D intoxication: Ingestion of increased amounts of vitamin D analogues, e.g. calcinogenic plants, dietary supplements, rodenticide baits, or other calcinogenic products are other possible causes of hypercalcemia.[5,6]
4) Certain types of *granulomatous disease* can lead to hypercalcemia, for example blastomycosis in dogs. Activated macrophages have the ability to synthesize the active form of vitamin D3, calcitriol.[5]
5) *Renal failure* (please see below): Renal failure is associated with phosphate retention (hyperphosphatemia). The increased serum phosphorus (PO4) forms complexes



4-1. Lung, dog. Diffusely, alveolar septa are necrotic and replaced by hyaline membranes, often admixed with scant amounts of granular mineral. Multifocally, alveoli are flooded with large amounts of fibrin, small amounts of hemorrhage, and few alveolar macrophages. (HE 400X)



4-2. Lung, dog. Diffusely within alveolar septa, there is mineral which stains positively with Von Kossa stain. (Von Kossa 400X) Photomicrograph courtesy of Department of Pathology & Infectious Disease, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, United Kingdom, www.rvc.ac.uk

with serum calcium (Ca2+) and metastatic tissue mineralization develops if the product of Ca2+ x PO4 exceeds a certain threshold value.[13]

In the present case, metastatic calcification was observed. In addition to the pulmonary mineralization, mineral deposition was present in the gastric mucosa, renal tubular epithelial cells and renal vessel walls. On macroscopic and microscopic examination of the dog and multiple tissues, the parathyroid glands were unremarkable and no evidence of neoplasia was found. The laboratory data indicated renal failure due to the concurrent presence of hypoalbuminemia, hyperphosphatemia and hyperkalemia and increased BUN and creatinine. The hypoalbuminemia was likely caused by renal loss of protein. The cause of the mild hypoglobulinemia is uncertain. No intestinal lesions were observed at post mortem examination. Hyperphosphatemia, hyperkalemia and increased BUN and creatinine develop following decreased glomerular filtration. The increased levels of amylase and lipase were also likely related to the renal disease, since no lesions suggestive of pancreatitis were observed. Interestingly, the dog also had hypercalcemia. In dogs with renal failure, there are usually hyperphosphatemia and hypocalcemia. In some cases, however, hypercalcemia has been described.[13] The elevated ALP and hyperbilirubinemia suggest decreased hepatic function. The young age of the dog may have contributed to the elevated calcium, phosphorus and ALP levels. Renal failure is classified as *pre-renal, renal* or *post-renal* renal failure. The main features of the different types of renal failure are listed below.

Pre-renal renal failure is related to a reduced perfusion of the kidneys, which can be caused by low cardiac output, increased renal vasculature resistance, extra-cellular fluid volume depletion or low systemic vascular resistance.[10]
Renal renal failure is caused by impaired renal function, which could be related to tubular injury, glomerulonephritis, tubulointerstitial nephritis, acute vascular nephropathy or neoplasia.[10]
Post-renal renal failure is caused by urinary tract obstruction, e.g. tubular precipitation, urethral obstruction or bladder obstruction.[10]

In this case, the absence of obstruction in the urinary tract rules out post-renal failure. Because of the history of vomiting and diarrhea, reduced renal perfusion has to be considered as a possible cause of the renal failure. Microscopic examination of the kidneys revealed the presence of mineralization in some tubular epithelial cells and in the walls of occasional blood vessels. Furthermore, mild tubular degeneration and regeneration were observed. The degeneration affected approximately 20-30% of tubules in the cortex and medulla. In addition, the renal interstitium contained a mild multifocal infiltrate of lymphocytes and plasma cells. Thus, pre-renal and renal causes likely contributed to the renal failure. Reportedly, vomiting and diarrhea develop prior to the acute renal failure. Vomiting and diarrhea, however, can also be observed secondary to renal failure.[5,10]

In secondary renal hyperparathyroidism raised levels of ionized plasma phosphate cause hypocalcemia through the precipitation of calcium and phosphorus. The reduction in ionized serum calcium stimulates PTH secretion and the mobilization of calcium from bone stores. This results in bone resorption with the release of phosphorus and calcium. The stimulation of PTH secretion is compounded by the damaged kidneys, which have a reduced capacity to hydroxylate the inactive form of vitamin D 25-hydroxycholecalciferol to the active form of vitamin D, i.e. 1,25-dihydroxycholecalciferol (calcitriol). Thus, the intestinal absorption of calcium is reduced. Calcitriol, which is also suppressed by hyperphosphatemia, normally inhibits PTH secretion. By reducing calcitriol production a physiological control on PTH secretion is removed and eventually parathyroid chief cell hyperplasia occurs, a process termed renal secondary hyperparathyroidism.[12]

PTH enhances calcium release from bone by activating osteoclast resorption through their indirect stimulation. Osteoclasts do not have a receptor for PTH, thus PTH binds to osteoblasts which stimulate osteoblasts to increase their expression of receptor activator of NFκB ligand (RANK ligand). RANK ligand binds to osteoclast precursors containing receptor activator of NFκB (RANK). The binding of RANK ligand to RANK stimulates these precursors to fuse and to form osteoclasts which ultimately enhances the resorption of bone.[3]

The biochemical abnormality characterized by elevated BUN and creatinine is termed azotemia and when this is associated with clinical signs and other biochemical abnormalities it is termed uremia.[10] Non-renal lesions of uremia include pulmonary edema and soft tissue mineralization. In uremia, pulmonary edema most likely occurs via two mechanisms a) mineralization of alveolar septae leading to diffuse alveolar damage, and b) uremic toxins causing increased permeability of alveolar capillaries with leakage of protein-rich fluid containing fibrin.[10] Histiocytic cells, including alveolar macrophages have a role in preventing the accumulation of native proteins within alveoli and thus their increased infiltration would be expected in response to proteinaceous edema containing fibrin. In addition, the mineral deposits stimulate a foreign body response causing histiocytic infiltration.[10]

The pathogenesis of soft tissue mineralization in the uremic animal is not fully understood, however, deposition of calcium phosphate mineral favors tissues with an internal alkaline compartment, principally gastric mucosa, kidneys, lungs, systemic arteries and pulmonary veins but other local factors such as tissue glycosaminoglycans may play a role.[7] Renal mineralization compounds the problem by causing further damage and thus a further reduction in glomerular filtration.

In the present case, the pulmonary lesions might be caused by uremia. Due to the concurrent presence of hyperphosphatemia and hypercalcemia, however, hypervitaminosis D cannot be ruled out.[5]

Leptospirosis has also been considered as one possible cause of concurrent elevated renal and hepatic parameters in dogs.[1,10] Canine infection is caused by *Leptospira (L.) interrogans* or *L. kirschneri* and many pathogenic serovars have been identified with *Leptospira canicola, icterohaemorrhagiae, grippotyphosa, pomona* and *bratislava* being the most commonly identified in dogs.[9,14] Following cutaneous or mucosal penetration, leptospires are disseminated via the blood to target tissues such as the kidneys, liver, spleen and central nervous system. The extent of damage to internal organs varies with the virulence of the organism and host susceptibility. In addition, some serovars show a predilection for the liver or kidney. The mechanisms by which leptospires cause disease are not well understood, and leptospiral lipopolysaccharide may play a role in inflammatory and coagulatory abnormalities that occur. Renal damage occurs following colonization and replication of the organisms in renal tubular epithelial cells, whereas histochemical studies have shown that the fundamental hepatic lesion is due to subcellular effects on enzyme systems.[9] In this case the standard serologic test, microscopic agglutination test (MAT), was performed however titers to all tested serovars were negative. It has been reported that in acute or peracute disease antibody responses have not yet developed and as such serological tests are not useful until 7 to 10 days after infection.[1,9]

AFIP Diagnosis: Lung: Pneumonia, interstitial, fibrinonecrotizing, acute, multifocal to coalescing **(Fig. 4-1)**, marked with edema, hemorrhage, hyaline membranes and alveolar septal mineralization **(Fig. 4-2)**

Conference Comment: Animals that die due to complications of acute renal failure and subsequent uremia often develop terminal pulmonary edema. The pathogenesis is not clear, but increased permeability of the alveolar capillaries is the most likely cause. Mineralization of alveolar and bronchiolar walls can also occur secondary to fatal renal disease. This is often referred to as uremic pneumonitis or uremic lung.[10]

In reviewing the clinical chemistry data from this case, the moderator emphasized to attendees that an increase in ALP may occur for a variety of reasons, e.g. cholestasis, steroid induction. Furthermore, there may have been increased activity of the bone isoenzyme of ALP due to the young age of the puppy in the case, or perhaps increased osteoclastic activity due to the suspected renal hyperparathyroidism. Most importantly, ALP is independent and non-predictive of hepatic function. Analysis of pre- and post-prandial bile acid concentration is the only direct method to assess hepatic function; albumin and BUN concentrations (if low) may provide indirect clues to the status of liver function.

The moderator and conference participants also prefer the use of the term "azotemia" versus "renal failure" in the otherwise well-crafted contributor section on pre-, renal and post-renal azotemia. Renal failure, defined as an increase in BUN/Creatinine and decreased urine specific gravity, is present in this case.

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