Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2015-2016

C o n f e r e n c e 23

7 May 2016

CASE I: G10-083313 (JPC 4003029).

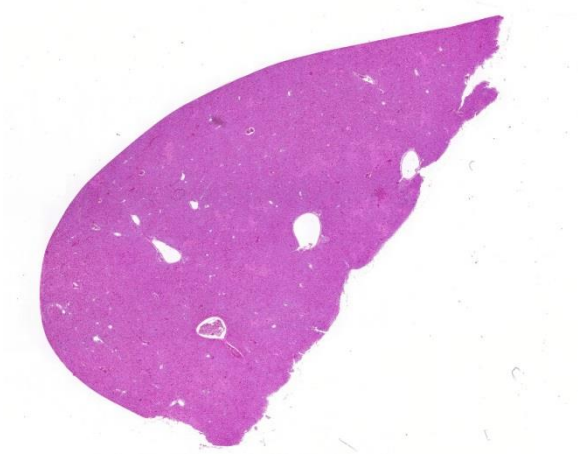
Signalment:
Flock of 20,000 11-week-old commercial meat turkeys, *Meleagris gallopavo.*

History: Flock experienced a spike in mortality. Flock is housed in barn and bedded with a layer of shavings on top of dirt floors. This is the first time this disease has been identified on this farm but the producer does have a second farm where turkeys are also raised and this disease has been a recurring problem on that farm.

Gross Pathology: The submitting veterinarian described swollen livers with yellow streaking and very enlarged dark spleens in the turkeys that were necropsied. Birds also had unclotted blood in the abdominal cavity.

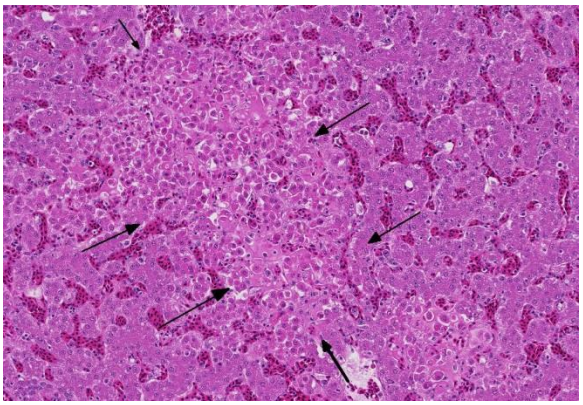
Laboratory Results: 4+ *Erysipelothrix rhusiopathiae* was recovered from a swab of the internal organs.

Histopathologic Description:



Liver, turkey. At subgross magnification, there are randomly scattered areas of pallor. (HE, 5X)

The liver is moderately congested. Multiple variably-sized foci of hepatic necrosis, characterized by individualized, hypereosinophilic hepatocytes with granular or shrunken, hyalinized cytoplasm and either lacking nuclei or containing pyknotic or karyorhectic nuclei, are closely associated with terminal hepatic veins. Hepatocytes surrounding these necrotic foci are occasionally swollen with pale vacuolated cytoplasm. Multifocally, veins and sinusoids and less frequently small arteries contain



Liver, turkey. Areas of pallor correspond to areas of necrosis (arrows) within which hepatocellular plate architecture is lost and necrotic hepatocytes are rounded up and brightly eosinophilic. (HE, 124X).

fibrin thrombi bearing mats of slender rod-shaped and sometimes gently curved bacteria. Similar appearing bacteria are also free in sinusoids and within activated Kupffer cells, which also contain phagocytized debris including red blood cells. Occasionally the walls of veins and small arteries containing fibrin thrombi have segmental necrosis, with some affected arteries having intramural heterophils and rarely small amounts of nuclear debris.

Contributor's Morphologic Diagnosis:
Liver: Mild, acute, multifocal, hepatic necrosis with necrotizing vasculitis and intravascular fibrin thrombi containing colonies of pleomorphic rod-shaped bacteria.

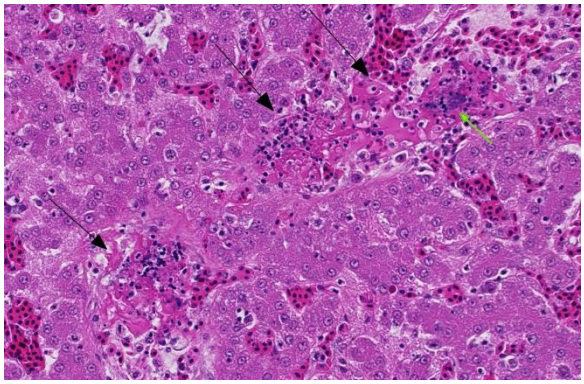
Liver: Moderate hepatic congestion

Contributor's Comment: Erysipelas is an acute septicemic disease occurring most commonly in older male turkeys. The differential diagnosis includes other gram-negative bacterial septicemias caused by agents such as *E. coli*, *Salmonella* spp. or *Pasteurella multocida*.[2] Histologically, the pathology of an *Erysipelothrix rhusiopathiae* infection is different from most gram-positive agents, first because of the sheer numbers of bacteria present and secondly because of their variable appearance, with slender rod-shaped to slightly curved bacteria aggregating in mats within vessel and capillary lumens and entangled in fibrinous thrombi. Because these bacteria are slow growing, a rapid presumptive diagnosis can also be made by identification of clumps of gram-positive slender straight or slightly curved rod-shaped bacteria from organ or bone marrow smears.[2]

This case was submitted to the lab in early October which is typical for cases of erysipelas, as outbreaks are reported to occur most often in the late fall or winter. It is thought that the bacteria can persist in the soil and since many grow-out barns for turkeys have dirt floors, the risk of repeat occurrences exists.[2] In this case, this farm has never experienced an outbreak of erysipelas but the other farm has and it is suspected that there was mechanical transfer of the bacterium from one farm to another.

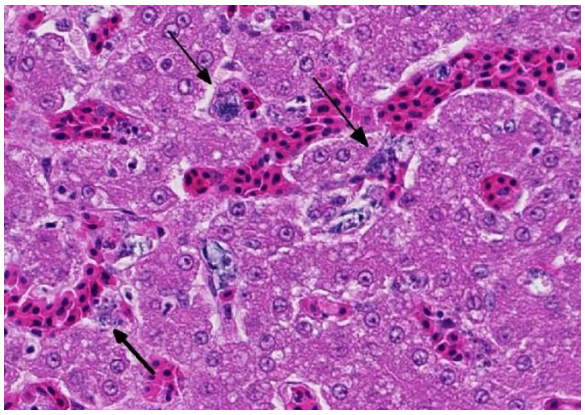
Penicillin is the antibiotic of choice for treating erysipelas. Vaccination using a killed bacterin is an option if the risk of infection is high.[2]

In humans, the infection caused by *Erysipelothrix rhusiopathiae* is known as



Liver, turkey. Septic thrombi (green arrow) are asscoated with vasculitis (black arrows) throughout the section. (HE, 150X)

erysipeloid, a skin infection typically localized to fingers and hands and usually preceded by an abrasion or cut. The lesion is actually a cellulitis and is very painful. Systemic effects, such as septicemia and endocarditis can occur but are uncommon.[6] Most cases of human infection are the result



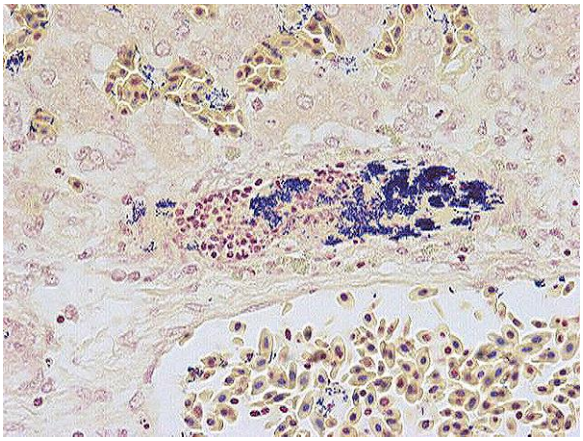
Liver, turkey. Kupffer cells contain ingested bacilli (arrows). (He, 260X)

of occupational exposure and those occupations at higher risk include fish handlers, veterinarians, farmers, slaughter plant workers and butchers. Some colloquial names for this condition include fish handler's disease, seal finger and whale finger.

JPC Diagnosis: Liver: Hepatitis, necrotizing, acute, multifocal, random, with septic fibrin thrombi and vasculitis.

Conference Comment: In addition to outbreaks in domestic turkeys, *E. rhusiopathiae* outbreaks have also been reported in laying hens in Europe,[3] and sporadically in a variety of other captive and free-ranging birds. The organism is fastidious and able to survive in the environment for extended periods. It may be transmitted by cuts and abrasions or through ingestion. It is generally considered to follow an acute course characterized by septicemia, but a chronic form also occurs in turkeys,[1] which appear to be most susceptible to infection. In addition to thromboembolism, bacterial endocarditis and joint infections[1] may be seen in affected turkeys, among other signs of septicemia. Thrombosis and hemorrhage are commonly reported in avian species infected with *E. rhusiopathiae*, reflecting the vasculocentric nature of the disease. Grossly, carcasses of affected birds are in good flesh and exhibit organomegaly of the liver, spleen and kidneys, as well as eccymotic hemorrhages in the subcutis and muscles.[1] Routes of infection include fomites, contaminated soil, insect vectors, asymptomatic carrier animals and contaminated feed.[3,4]

Although it has been reported, infection in psittacine birds is considered rare. In a case report of infection in a mixed species aviary, lesions included thrombosis, bacterial thromboembolism, necrotizing hepatitis, necrohemorrhagic myocarditis, and hemorrhage.[4] *E. rhusiopathiae* infection has also been reported in emus, which are large flightless birds that are grouped with other ratites such as ostriches and rheas. Lesions similar to those reported in other species are also seen in emus, including hepatocellular necrosis with absence of an abrupt



Liver, turkey. Gram-positive bacilli are present within septic thrombi. (Photo courtesy of: Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada, http://ahl.uoguelph.ca)

inflammatory response. Bacteria may be observed in multiple organs, including the kidneys and small intestine as well as the liver; the presence of fibrin thrombi, while prominent in many cases, may be variable.[5]

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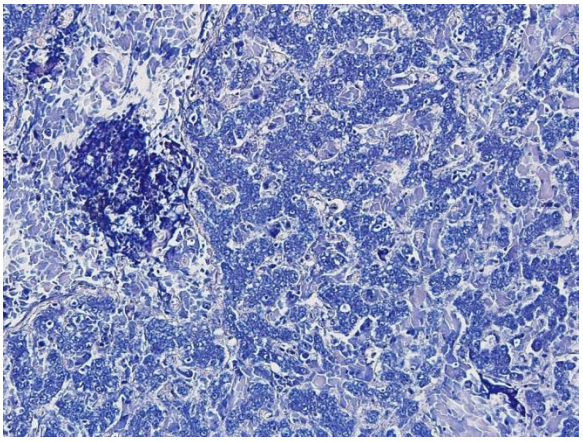

Liver, turkey: A phosphotungstic acid hematoxylin (PTAH) stain demonstrates fibrin thrombi within vessels (left arrow) and within hepatic sinusoids (right arrow). (PTAH, 200X)

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CASE II: UFMG-2 (JPC 4035761).

Signalment:
Male, Coimbra's titi (*Callicebus coimbrai*)

History: This monkey was kept at the zoological park in Belo Horizonte, Brazil. It was found on the floor in a cage, prostrated and hypothermic. It received emergency therapy with fluids, corticosteroids, glucose, and heating, but died soon after the initial treatment.

Gross Pathology: Grossly, the mesenteric lymph nodes were hemorrhagic, the colon was dilated with multifocal and moderate hemorrhage in the serosa. The colon content was hemorrhagic with a few blood clots, and large amounts of fibrinous exudate. There was sand in the oral cavity, esophagus, and stomach. The heart was mildly dilated. On the surface and cut surfaces of the liver, there was a prominent lobular pattern. Kidneys and adrenal glands were moderately congested.

Laboratory Results: None.

Histopathologic Description: Multifocal to coalescing and severe necrosis associated to

Colon, titi monkey. Colonic contents were hemorrhagic, with clumps of fibrin. (Photo courtesy of: Universidad Federale de Mias Gerais. Avenida Antonio Carlos, 6627. PO Box. 567. Setor de Patologia. Departamento de Clinica e Cirurgia Veterinarias. Escola de Veterinaria. Belo Horizonte, Minas Gerais. Brazil. 31270-901.)

multifocal and moderate hemorrhage was observed in the mucosa of the colon. Myriads of round, 30-50 µm diameter, eosinophilic staining trophozoites were diffusely distributed in the lumen, necrotic mucosa, crypts, and lamina propria of the colon. There was a multifocal and mild infl-ammatory infiltrate composed of ly-mphocytes, histiocytes and neutrophils in mucosa and submucosa. Some epithelial cells of the colonic crypts had numerous circular, intensely basophilic cytoplasmic structures, which were interpreted as apoptotic bodies.

Contributor's Morphologic Diagnosis: Large intestine, colon: Colitis, necrotizing, hemorrhagic, acute, multifocal to co-alescing, severe with numerous protozoal organisms consistent with *Entamoeba histolytica*.

Contributor's Comment: *Entamoeba histolytica* is a protozoan parasite capable of invading the intestinal mucosa. It affects other organs, mainly the liver, causing amebiasis.[7] *Entamoeba dispar* is mor-phologically similar. It also colonizes the human gut, however it has no invasive potential.[1] Both species are found of non-human primates, such as monkeys, orangutans, and baboons.[5]

Most asymptomatic infections found worldwide are now at-tributed to *Entamoeba dispar*, because it is non-invasive. *E. dispar* is distinct but closely related to *E. histolytica*. This non-invasive species has im-plications for understanding the epidemiology of amebiasis.[3]

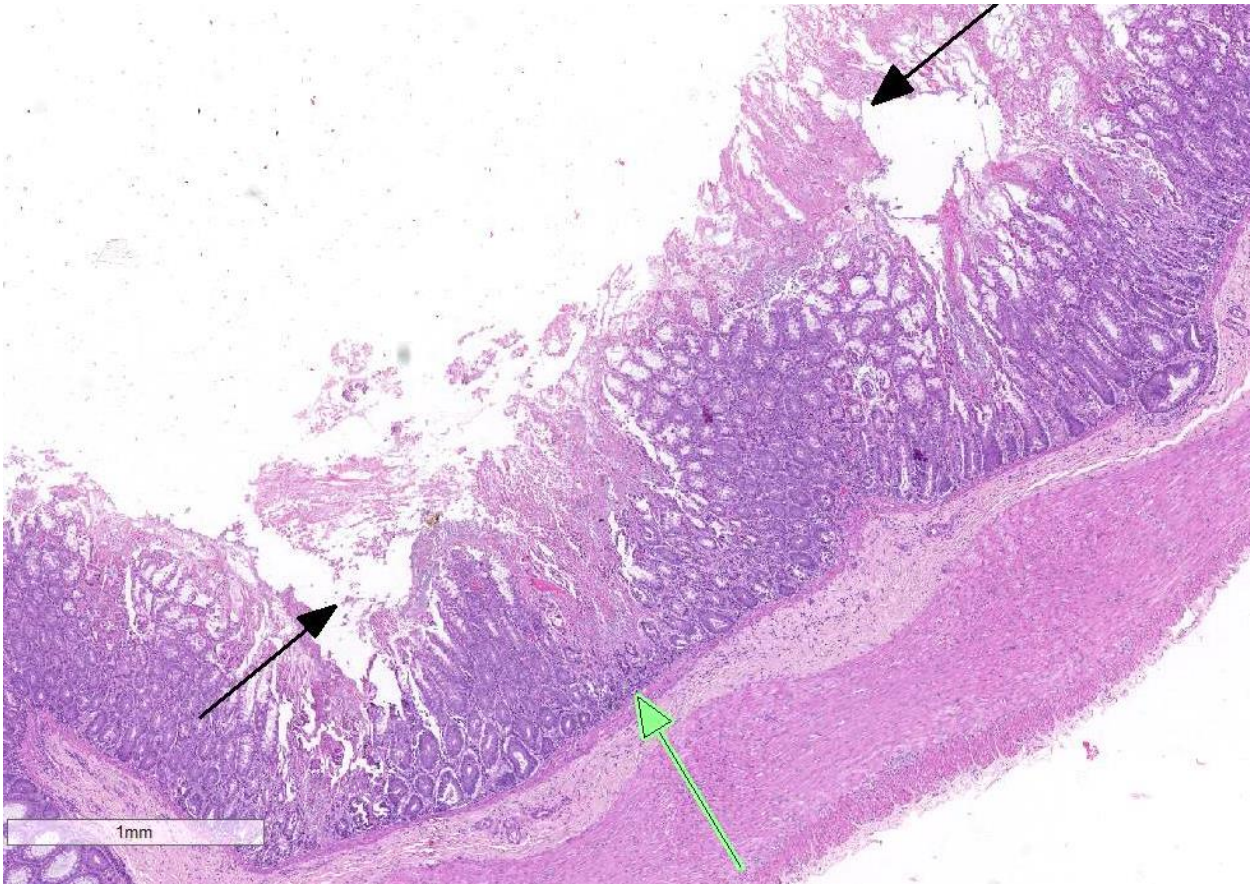
In humans, amebiasis is more common in developing countries. Bad conditions such as overcrowding, poor education, co-ntaminated water, and poor sanitation favor fecal-oral transmission of amoebas. This disease results in 70 thousand deaths annually. Amebiasis is considered the fourth cause of death due to protozoa.[11] A recent study revealed a high prevalence of *E. histolytica* in long-tailed macaques in the Philippines.[8] Captive monkeys infected with *E. histolytica* is a concern not only for the animal health risk, but also due to the zoonotic nature of the disease.[4]

The trophozoite, which is the motile form of *E. histolytica*, lives in lumen of crypts in large intestine, where it multiplies and differentiates into cyst (resistant form responsible for transmission). Cysts are excreted in stools, and may be ingested by a new host through contaminated food or water. Upon ingestion of infective cysts, parasites are released in terminal ileum, with each emerging quadrinucleate trophozoite giving rise to eight uninucleated trophozoites. Trophozoites may invade the colonic mucosa and cause dysentery.[3]

There are some molecules produced by *E. histolytica* that are related to lysis of the colonic mucosa: adhesins, amoebapores, and proteases. The parasite attachment to colonic mucus blanket is due to a multifunctional adherent lectin, preventing elimination in intestinal stream. Lectins are involved in signaling cytolysis and in blocking the deposition of the injurious membrane attack complex of complement. It maybe also participates in anchorage of the ameba to extracellular matrix during invasion and contribute in the lysis of target cells.[3]

JPC Diagnosis: Colon: Colitis, necrotizing, acute, multifocally extensive, marked with crypt abscesses, goblet cell loss and numerous amoebic trophozoites.

Conference Comment: *E. histolytica* colonizes the large intestine resulting in

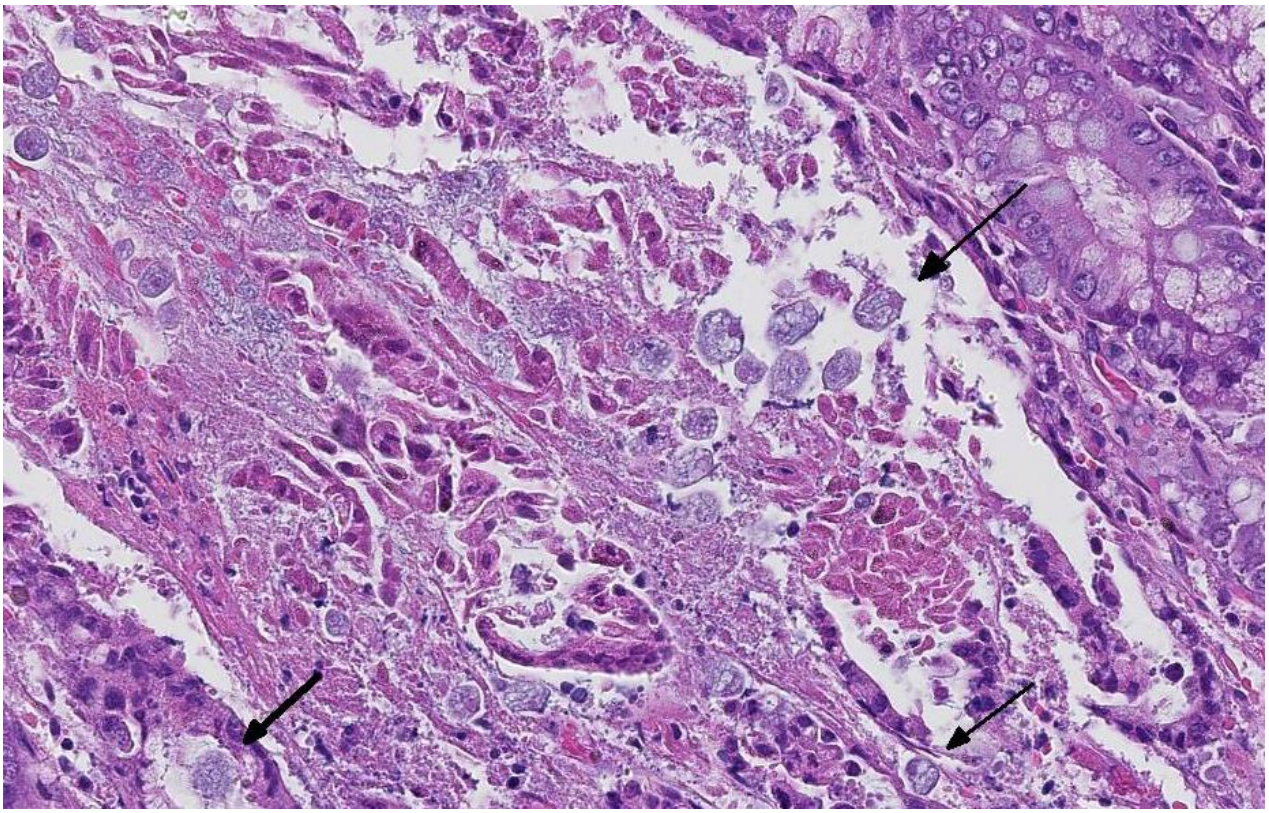


Colon, titi monkey. The proximal 33% of the mucosa of the colon exhibits coagulative necrosis (black arrows), with occasional full thickness necrotic areas perpendicular to the mucosal surface (green arrow). (HE, 18X)

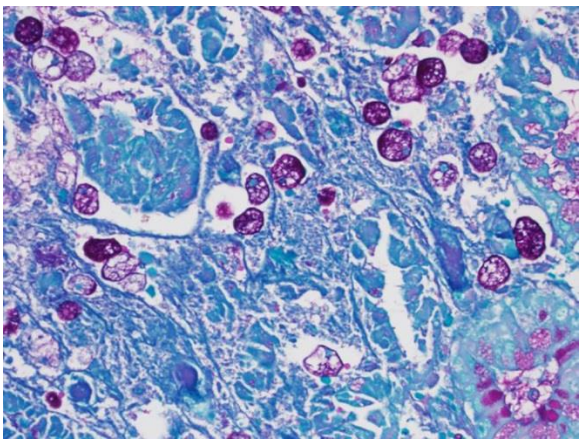
proteoglycans, during invasion process.[3] Peptides of *E. histolytica* named amoebapores destroy phagocytosed bacteria from the microbiota that serve as the main nutrient source for the parasite. Whether amoebapores play a role in the cytolytic event has not yet been proven. Proteases produced by the parasite can degrade the colitis and diarrhea, and in some cases, may result in liver abscesses. *E. histolytica* has two morphogenetic forms: the trophozoite and infectious cyst form. Ingestion of viable cysts initiates infection and subsequent steps are discussed above. The initial attachment to mucosal enterocytes by trophozoites, mediated through adhesins and lectins,

appears to be important in the initial pathogenesis and apparently plays a role in cytotoxic activity.[6] The host inflammatory response contributes to tissue damage and may help facilitate infection. Important cytokines involved in the host response include IL-8, IL-6 and IL-1α; genes involved in cell proliferation also participate in the response to infection. *E. histolytica* destroys host tissue and commonly causes enterocyte apoptosis; trophozoites may ingest host cells following their death. The mechanisms leading to host cell apoptosis are not completely understood, but may involve production of reactive oxygen species and oxidative stress. Other mechanisms which may be involved in cell death include amoebic trogocytosis, where the parasite ingests fragments of host cell, resulting in increases in intracellular calcium and cell death. [2] Other parasite factors which are involved in the pathogenesis include production of prostaglandin E2, which increases sodium ion permeability through tight junctions, as well as secretion of cysteine proteases which digest matrix components such as gelatin, collagen type I and fibronectin. Epithelial barrier disruption also plays an important role in infection.[2]

E. histolytica trophozoites normally remain in the colonic lumen. However, in some cases, the trophozoites invade the mucosa as well as mural blood vessels and lymphatics, and eventually infect the liver. Ulcerative gastritis secondary to *E. histolytica* infection has also been described in primates that have a sacculated stomach, which is adapted for leaf eating and fermentation. Similarly, *E. histolytica*-associated gastritis may also occur in macropods (kangaroos and wallabies), which likewise have a sacculated



Colon, titi monkey. Necrotic areas within the mucosa contain numerous 15-20um amebic trophozoites. (HE, 324X)



Colon, titi monkey. Amebic trophozoites stain intensely with periodic-acid Schiff stains (an excellent choice for amebae.) (PAS/Alcian blue counterstain, 400X)

stomach. [9] Trophozoites are observed in the mucosa and gastric glands, but may also invade to the level of the submucosa, including vessels and lymphatics.[9]

E. histolytica may also cause necrotizing and ulcerative colitis in dogs and cats (likely acquired from a human source) although less commonly than in primates. In cats, *E. histolytica* infection has been associated with severe necrosis of the colon and cecum.[9,10] It is apparently uncommon for infected dogs to shed infectious cysts.

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Escola de Veterinaria.
Universidade Federal de Minas Gerais.
Belo Horizonte, Minas Gerais.
Brazil

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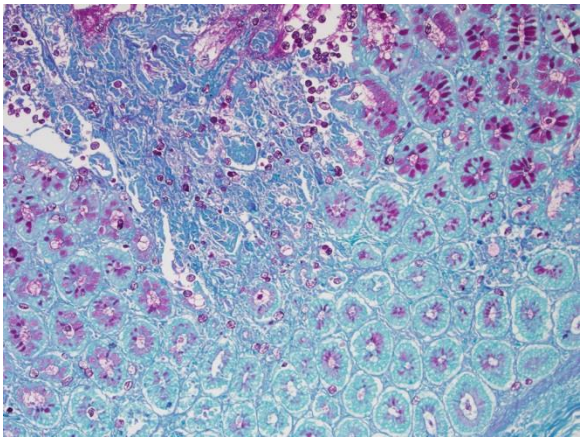

Colon, titi monkey. The infiltration of amebic trophozoites into the mucosa causes a downregulation of mucin production by nearby goblet cells. (PAS/Alcian blue counterstain, 100X)

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Figures

Figure 1. Colon, *Callicebus Coimbrai*: hemorrhagic content.

Figure 2. Colon, *Callicebus Coimbrai*: severe necrosis associated to eosinophilic staining trophozoites consistent with *Entamoeba histolytica* (HE 200X).

Figure 3. Colon, *Callicebus Coimbrai*: myriads of round, 30-50 µm diameter, eosinophilic staining trophozoites consistent with *Entamoeba histolytica* in crypts and the lamina propria (HE 400X).

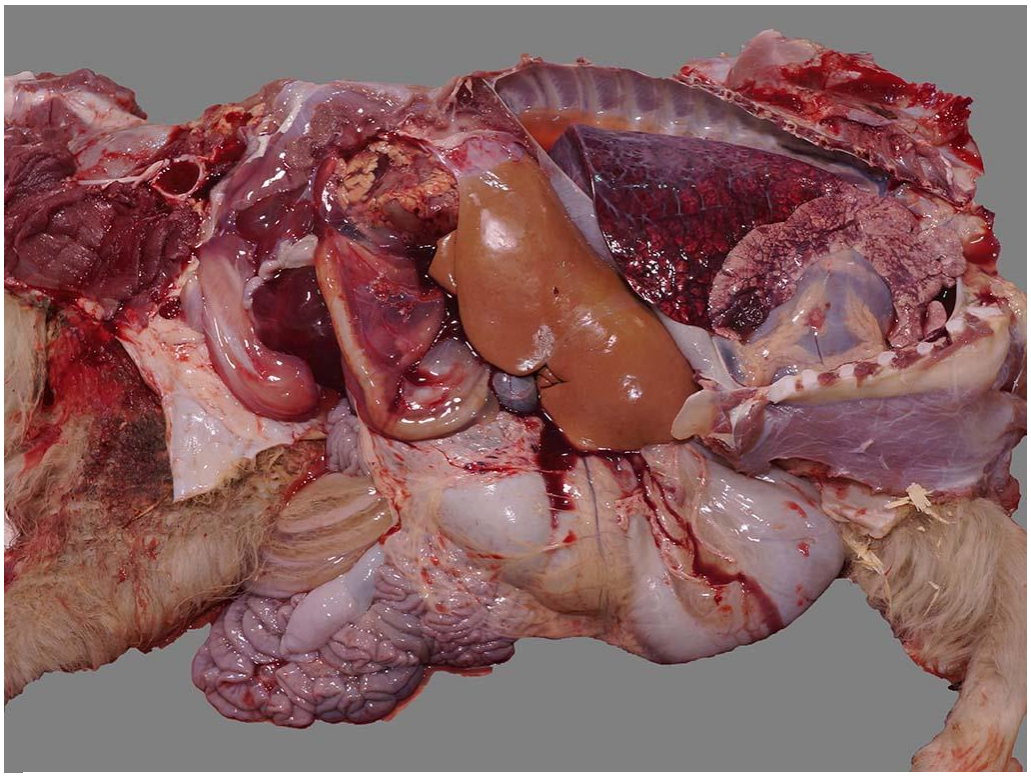
Figure 4. Colon, *Callicebus Coimbrai*: myriads of round, 30-50 µm diameter, eosinophilic staining trophozoites consistent with *Entamoeba histolytica* in crypts and the lamina propria (PAS 400X).

CASE III: D14-043814 (JPC 4066096).

Signalment: 2.5 month old, intact female, Yak (*Bos grunniens)*

History: This yak was bottle-raised from birth and housed in a small pen in the backyard, abutting a building. The yak had an approximately two week history of pica and a one week history of decreased appetite and lethargy, which progressed to loss of a suckle response. The animal developed a fever (104F) and was initially treated with antibiotics and tube feedings of electrolytes and milk at the farm but was referred for suspected laryngeal or pharyngeal trauma based on difficulty tubing and reflux of milk through the nose. On presentation to the referral hospital, the yak was tachypneic (112 rpm) with harsh lung sounds bilaterally and decreased lung sounds ventrally. The yak was markedly azotemic (results below) and was started on intravenous fluids and other supportive care. Thoracic radiographs supported cranioventral pneumonia and a trans-tracheal wash was performed (results below) and antibiotics were initiated. The yak's azotemia did not improve with fluid therapy and the yak was producing minimal urine. The yak developed ascites and a total of 4L was removed via abdominocentesis on the two days preceding euthanasia. The yak did not suckle at any time during hospitalization. The yak was euthanized 7 days after admission to the referral hospital.

Gross Pathology: The abdominal cavity contained 2.5 L of translucent, pale pink to yellow, watery fluid and there was marked perirenal edema with mild to moderate hemorrhage, more pronounced surrounding the left kidney. The kidneys were diffusely pale brown and slightly swollen, with small pinpoint foci of hemorrhage in the cortex, reddening of the corticomedullary junction, and hemorrhage and edema in the adipose tissue of the renal pelvis. There was also



Kidney, yak calf. There was marked perirenal edema with mild to moderate hemorrhage, more pronounced surrounding the left kidney. The liver was diffusely a pale brown. The lungs were red in caudal lobes with interstitial edema, and the cranioventral fields were firm as a result of aspiration pneumonia. (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

marked mesocolonic edema and mild to moderate generalized subcutaneous edema. There were no lesions in the esophagus, pharynx or larynx. The rumen contained a moderate amount of gray opaque liquid and small chips of gray paint. The liver was diffusely pale brown. The thoracic cavity contained 0.3 L of fluid and the pericardium contained 0.1 L of fluid, similar to the abdominal fluid. The lungs were dark red caudally and mottled pink and dark red cranially with diffuse interstitial edema and multiple dark red, firm areas of consolidated lung bilaterally in the cranioventral lung fields, consistent with aspiration pneumonia (foreign material present on histopathology).

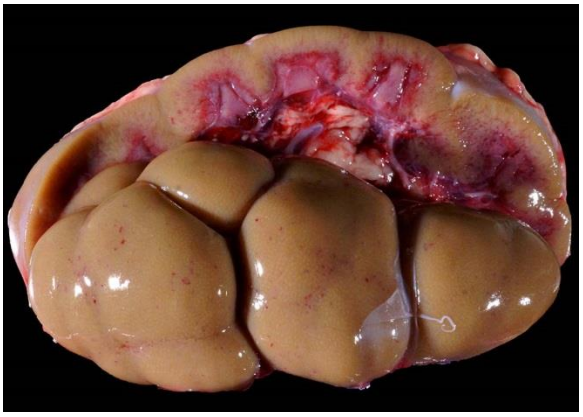
Laboratory Results:

Clinical pathology:

*Reference intervals were not provided for yak; references ranges in parentheses are for cattle tested at the referral hospital for general comparison only

<u>Significantly abnormal values on presentation</u>:
BUN – 128 mg/dL (10-24)
Creatinine – 17.1 mg/dL (0.6-1.3)
Calcium – 7.6 mg/dL (8.1-10)
Phosphorus – 11.6 mg/dL (3.4-7.7)
Total protein – 4.7 mg/dL (6.2-8.9)
Albumin – 2.8 mg/dL (3.2-4)
HCT – 18% (25-47)

<u>Values on the day of euthanasia (7 days later)</u>:
BUN – 119 mg/dL
Creatinine – 20.1 mg/dL
Calcium – 8.2 mg/dL
Phosphorus – 10.2 mg/dL
Total protein – 3.4 mg/dL
Albumin – 1.9 mg/dL
PCV – 12%



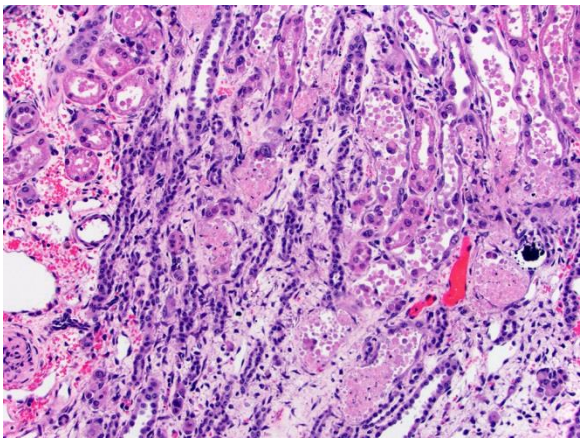
Kidney, yak calf. The kidneys were diffusely pale brown and slightly swollen, with small pinpoint foci of hemorrhage in the cortex, reddening of the corticomedullary junction, and hemorrhage and edema in the adipose tissue of the renal pelvis (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

Microbiology:
Antemortem aerobic culture of trans-tracheal wash: *Pseudomonas aeruginosa*
Postmortem aerobic culture of lung: *Pseudomonas aeruginosa*
Postmortem aerobic culture of kidney, spleen and liver: No growth

Molecular diagnostics:
Kidney was submitted for *Leptospira* species PCR (16s rRNA): negative
Tissue homogenate of lung and spleen was submitted for BVD PCR: negative

Toxicology:
Liver was submitted for toxic element screen:
Lead detected at 62 ppm (wet tissue basis) (<1 ppm considered normal for cattle, >10 ppm liver lead is consistent with toxicosis)

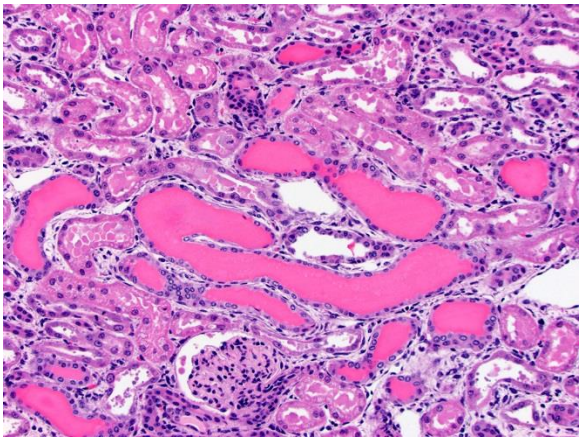
Histopathologic Description: Kidney – Diffusely affecting the entire cortex, the proximal tubular epithelium is degenerate or necrotic with marked clear cytoplasmic vacuolization (degeneration) of the proximal convoluted tubule (PCT) segments. The PCT have cuboidal epithelium with a discernible brush border and uniform, basally located nuclei frequently containing aggregates of 2-5 micron diameter, eosinophilic globular material displacing but not marginating the chromatin. Within the PCT lumina, there is eosinophilic material. The plump vacuolated cells of the PCT transition to the proximal straight tubule (PST) segments, which are lined by variably cuboidal to flattened epithelium, often with large nuclei (up to 50 microns in diameter or approximately 3-4 times the size of a normal renal tubular cell) and occasionally multiple nuclei, which contain similar eosinophilic intranuclear material as well as mitoses (interpreted as mixed degeneration and regeneration). There are areas of tubular epithelial necrosis and loss within the PST segment which is characterized by exposure of the basement membrane and numerous cells with pyknotic or karyorrhectic nuclei which are sloughed into the tubular lumen, admixed with small amounts of eosinophilic globular material. There are minimal histologic changes discernible in the



Kidney, yak calf. There are is extensive necrosis of tubular epithelium lining the proximal straight tubule. (HE, 100X) (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

medullary components (Loops of Henle, collecting ducts). The distal straight and convoluted tubules (DST, DCT) have uniform cuboidal epithelium with slightly

basophilic cytoplasm, central located nuclei which rarely have eosinophilic intranuclear material but moderate anisokaryosis, no discernible brush border and often have homogeneous pale to brightly eosinophilic material in the tubule lumen (high protein-



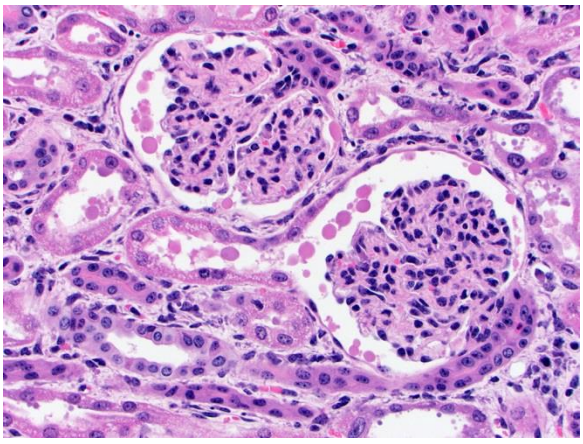
Kidney, yak calf. Distal tubules contain bright pink eosinophilic protein within their lumina. (HE, 200X) (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

content fluid). Occasionally, there are small aggregates of basophilic granular material within the tubular lumina (mineral). The glomeruli are condensed and occasionally have slightly increased pale eosinophilic material expanding the mesangium. The interstitium of the renal cortex is expanded by clear to amphophilic, occasionally granular material (edema fluid) and mildly increased amounts of immature collagen and fibroblasts (fibrosis). There are multiple nodular aggregates of moderate numbers of lymphocytes, smaller numbers of numbers of plasma cells and macrophages within the cortical interstitium. There are multiple small areas of hemorrhage within the perivascular space, interstitium, fibroadipose tissue subjacent to the renal pelvis, and renal capsule. Occasionally the aggregates of eosinophilic intra-epithelial, intra-nuclear material were strongly acid-fast positive and frequently were moderately PAS positive.

Contributor's Morphologic Diagnosis:

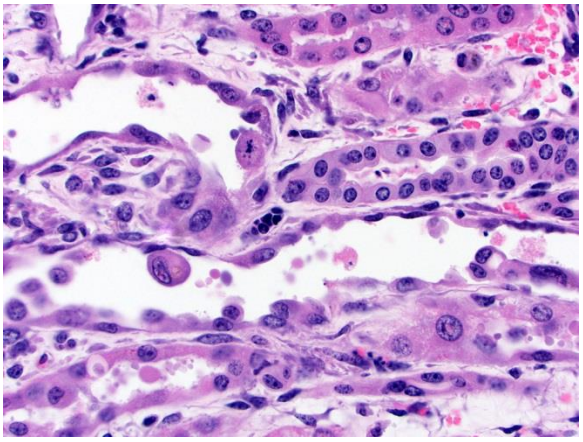
1) Kidney, tubules – tubular degeneration and necrosis, diffuse, marked, acute, with intra-epithelial intra-nuclear eosinophilic material (occasionally acid-fast and frequently PAS positive), tubular regeneration, and interstitial edema and hemorrhage
2) Kidney – nephritis, interstitial, lymphocytic, multifocal, mild, chronic

Contributor's Comment: This case is an example of acute tubular necrosis in a milk-fed, domestic yak calf (*Bos grunniens*) due to acute-to-subacute lead toxicosis. This animal was exposed to lead by ingestion of lead-based paint (gray paint chips in the rumen) chewed from the siding of the adjacent building. The clinical presentation of ruminants with lead toxicosis is often one of neurologic deficits or altered mentation. Histologically, there may be cerebral edema and laminar necrosis;[9] however, this yak did not have gross or histologic evidence of notable cerebral edema or necrosis and, other than lethargy and an inability to suckle, was not reported to have neurologic



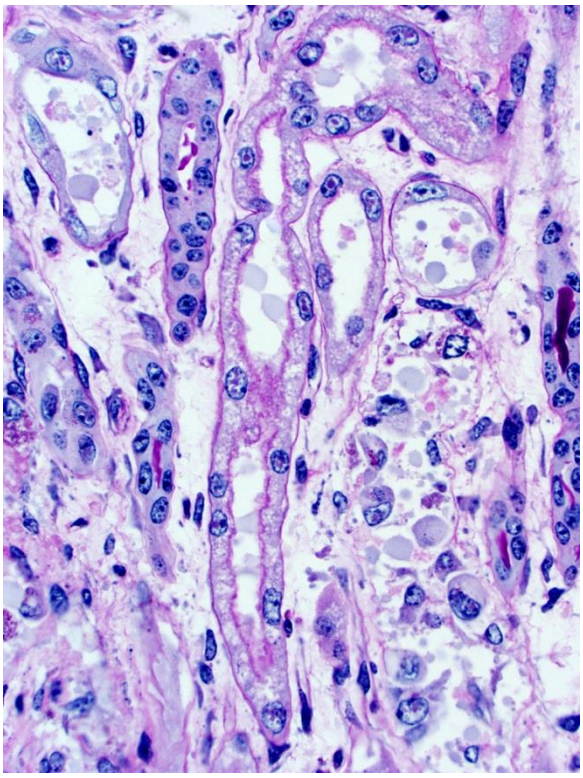
Kidney, yak calf. Refluxed protein is present within Bowman's space as well as within the proximal segment of the proximal convoluted tubules. (HE, 400X) (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

deficits. Peripheral neuropathy, including dysphagia and laryngeal paralysis, has been reported to be a common clinical sign in horses with both acute and chronic lead toxicosis[11] and may be the underlying reason for this yak's reported loss of suckle, reflux and aspiration pneumonia which resulted in referral.



Kidney, yak calf. Tubular epithelium contains evidence of necrosis and regeneration including sloughed epithelium and attenuated to plump, often layered epithelium which contains mitotic figures. (HE 600X)(Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

Renal changes attributed to lead toxicosis are usually reported to be mild and occasionally evident only as eosinophilic or poorly-staining intranuclear inclusions which are acid-fast positive but, similar to this case, severe nephrosis has also been described in young calves.[9] The mechanism of renal toxicity of lead is known to involve numerous pathways. Structural mitochondrial degeneration occurs (mitochondrial swelling and distortion of cristae) as well as decreased activity of mitochondrial heme-pathway enzymes,[5] which interfere with cellular energy production needed to maintain homeostasis and fuel active transport processes vital to renal tubular cell function. Lead also impacts nuclear function through altered gene expression[5] and is histologically and ultrastructurally evident as lead-protein inclusion bodies in some cases. Interestingly, not all of the histologically-

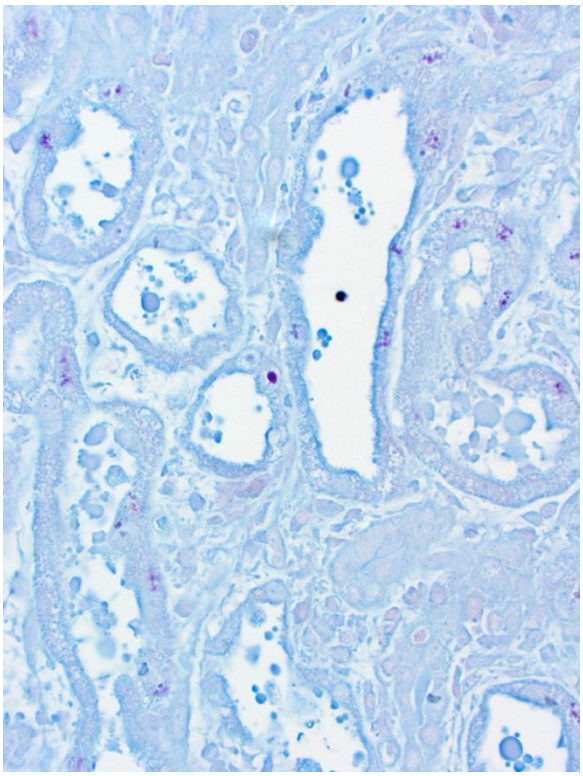


Kidney, yak calf. Nuclei of tubular epithelial cells often contain PAS-positive inclusions. (PAS, 200X) (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

evident eosinophilic intranuclear material in this case was acid-fast positive but the majority was variably PAS-positive, suggesting that many of the inclusions are high in glycogen or other carbohydrate-rich compounds but did not necessarily contain lead complexes. Electron microscopy was performed in this case and solitary or multiple, variably-sized electrodense inclusions were present in the nuclei of some renal tubular epithelial cells, however the inclusions incorporated less fibrillar material than expected based on previously published reports of lead inclusions.[3,12] This may be due to the fact that this was a diagnostic case and the tissue was formalin-fixed prior to gluteraldehyde fixation, which may have introduced artifactual changes. Additional

information regarding the composition of inclusions could have been obtained from energy-dispersive x-ray spectroscopy (EDS) but this capability is not available within the electron microscope at the submitting institution. The specificity of renal acid-fast positive inclusions for lead intoxication has been reported to be high in cattle[13] but renal intranuclear acid-fast inclusions should be further investigated. Other general mechanisms of lead toxicity likely impacting renal function include lead competitively binding in place of calcium, altered calcium regulation, and structural and functional alteration in cellular enzymes.[10]

Young animals are known to absorb ingested lead more efficiently than adults[7] and there are numerous dietary factors known to impact absorption of ingested lead.[1] Many studies on lead absorption are rodent-based; however, studies in cattle identified significant differences in lead absorption from milk-fed vs. grain and hay-fed calves[15] and in absorption based on levels of lactose in the diet.[14] Calves fed exclusively milk and calves fed elevated lactose levels with grain absorbed more lead than calves not receiving milk or without high levels of lactose supplemented to grain. These diet-related factors may be a major factor for susceptibility in young animals, and in this case the affected yak was on a milk-only diet.



Kidney, yak calf. Inclusions within nuclei of tubular epithelium are often acid-fast. (Ziehl-Nielsen, 100X) (Photo courtesy of: University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu)

The underlying cause and significance of the lymphocytic interstitial nephritis in this case is unknown.

JPC Diagnosis: 1. Kidney: Tubular degeneration, necrosis, regeneration, and proteinosis, diffuse, marked with tubular casts and intranuclear, eosinophilic inclusion bodies within renal tubule epithelial cells.

2. Kidney: Nephritis, cortical, interstitial, chronic, multifocal, mild.

Conference Comment: In addition to domestic animals, wildlife may also be exposed to lead from various sources. Ingestion of lead is the most common route of exposure, although toxicity due to lead-containing shot is also common (but likely poses a low risk for lead toxicity).[8]

Lead exposure is of particular concern in wild avian species where it may affect a variety of birds ranging from waterfowl to bald eagles. In avian species, lead can inhibit enzymes involved in hemoglobin synthesis and when exposed at high levels, may result in anemia. Lead toxicity is most often a chronic condition and as such, affected birds are often debilitated and in poor body condition.[6] Gross lesions may include esophageal, ventriculus and pro-ventriculus impaction with food, gall bladder distension, pale streaks in the

myocardium and muscle of the ventriculus indicative of necrosis, as well as pallor of internal organs. Eosinophilic lead inclusions in the nuclei of proximal tubule epithelium also occur in the kidney of birds and, while this finding is specific for lead poisoning, it may not be present in all cases. Although not necessarily specific for lead toxicity, other histologic changes in affected birds may include hepatic hemosiderosis, fibrinoid necrosis of arterioles, encephalopathy and peripheral neuropathy. The highest concentration of lead in birds is found in the bone, liver and kidney; lead levels in bone decline much slower than soft tissues and thus bone serves as a much longer term location of lead deposits.[6]

Lead has no biologic function and although lead can exert its toxic effects via a variety of mechanisms, its competition with calcium in various biologic functions is of particular importance. This can result in various pathophysiologic effects such as inhibition of neurotransmitter release, defects in ion pumps and channels as well as alterations in protein kinase function.[6] Lead is considered neurotropic, although the precise reason is not well understood; neuronal changes are non-specific and laminar cortical necrosis may be seen in some cases.[2]

In addition to the central nervous system, lesions also occur in bone. The characteristic lesion is referred to as a "lead line," which is a band of sclerosis located at the metaphysis of developing bones. It is seen as an early lesion in both children and animals. The lesion consists of persistent mineralized cartilage trabeculae which cannot be effectively resorbed by osteoclasts in spite of their apparent abundance microscopically. Osteoclasts may also contain acid-fast intranuclear inclusions.[4]

We thank the contributor for providing clinical pathology data and gross images with the submission, which enhance the teaching / learning value of the case.

Contributing Institution:
University of Minnesota – Veterinary Diagnostic Laboratory: www.vdl.umn.edu

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CASE IV: PA5050 (JPC 4033979).

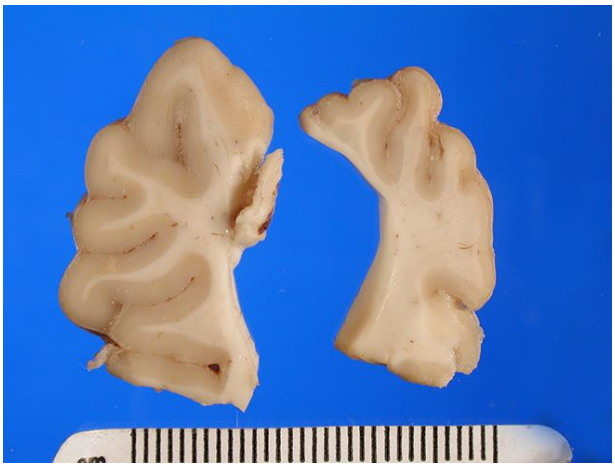
Signalment: 5-year-old, female, Spanish-Boer cross goat (*Capra hircus)*

History: This animal was noted to be acutely disoriented, visually impaired, and intermittently down and twitching. It responded immediately to empirical treatment, but never completely recovered full neurological function and was euthanized two months later.

Gross Pathology: The brain appeared somewhat shrunken on removal from the cranial vault. The cerebral cortex was thinned and discolored, especially in the occipital-parietal region, with areas of clefting and separation from the underlying white matter noted.

Laboratory Results: None

Histopathologic Description:
Sections of occipital-parietal cortex are examined. Each slide contains tissue from the affected animal as well as location-matched cortex from an age-matched control goat. The latter is essentially normal brain for comparison purposes. In the former, there is focally extensive laminar loss of cortical grey matter, significantly diminishing cortical thickness. Although occasional neuronal cells remain present, residual cellularity consists primarily of large, reactive (gemistocytic) astrocytes, activated microglial cells, and phagocytically active macrophages (Gitter cells). Capillary structures are prominent with somewhat swollen endothelium, both in grey



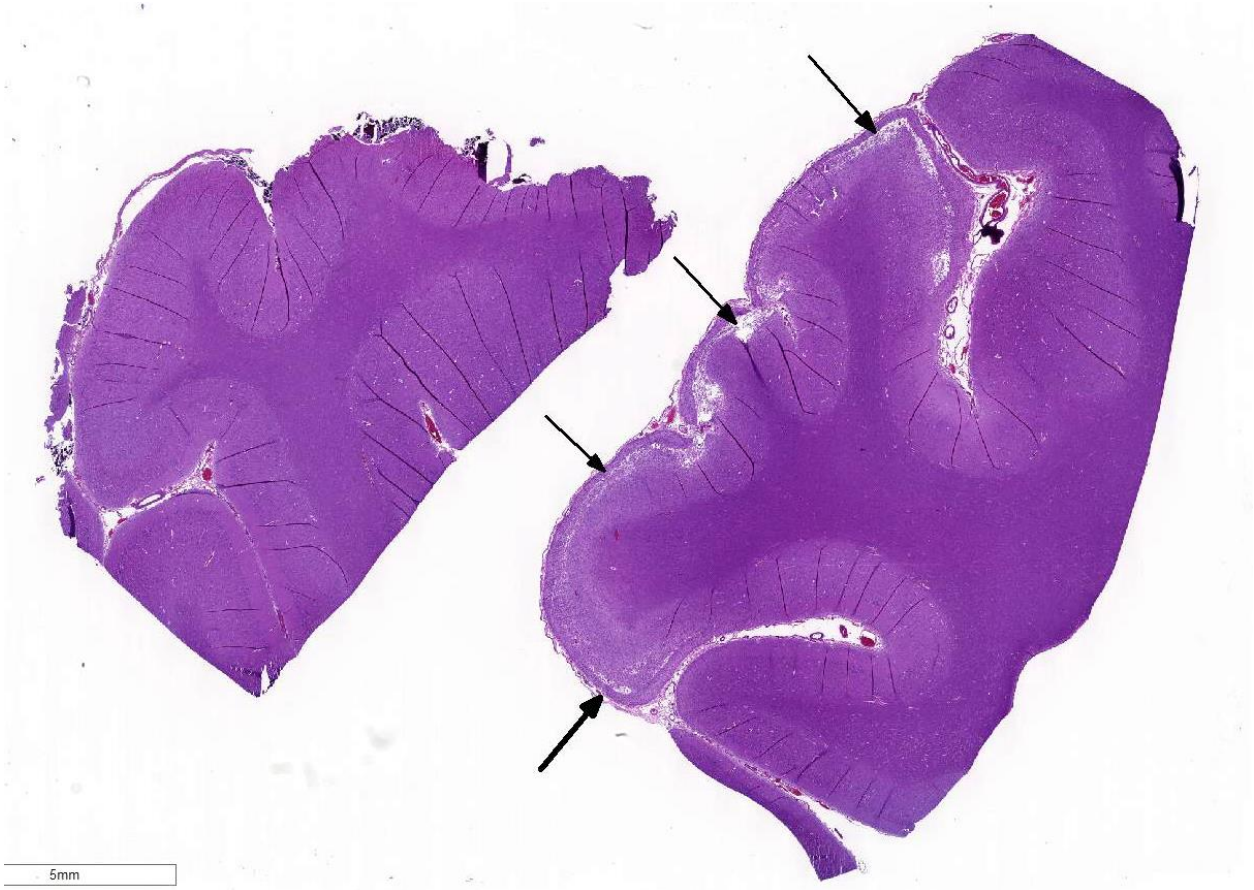
Cerebrum, goat: The cerebrum (at right) is shrunken with marked thinning of the gray matter and blurring of the interface between gray and white matter. (Age and location-matched cerebrum at left.) (Photo courtesy of: Division of Laboratory Animal Resources (DLAR) University of Pittsburgh, http://www.dlar.pitt.edu/

matter and in collapsed, redundant leptomeninges present in the expanded subarachnoid space. Abundant, phagocytically active macrophages are also noted in the latter. The white matter appears slightly hypercellular, probably due to a mild reactive astrocytosis also present in this region. Infrequent pyknotic cells, probably representing necrotic oligodendroglia, are seen due to axonal die back. Mild perivascular lymphocytic cuffing is noted in white matter in some sections.

Contributor's Morphologic Diagnosis:
1) Subtotal laminar cortical necrosis and collapse, severe, with marked extensive residual reactive gliosis, vacuolization and patchy regions of parenchymal separation/clefting, with areas of tissue dropout and meningeal collapse

2) Patchy, mild perivascular lymphocytic cuffing, subjacent white matter, mild (some sections)

Contributor's Comment: The microscopic findings are consistent with polioencephalomalacia (PEM). This is a morphological term used to describe necrosis with softening (malacia) in grey matter of the brain. The condition is described in a surprisingly wide range of domestic animals, both ruminant and carnivores, as well as some non-domestic species.[9] Wernicke's encephalopathy is the equivalent human disease, which is classically associated with chronic alcoholism. Cattle, sheep and goats are commonly affected ruminants, although a variety of other species are also susceptible. Clinical manifestations of the disease are

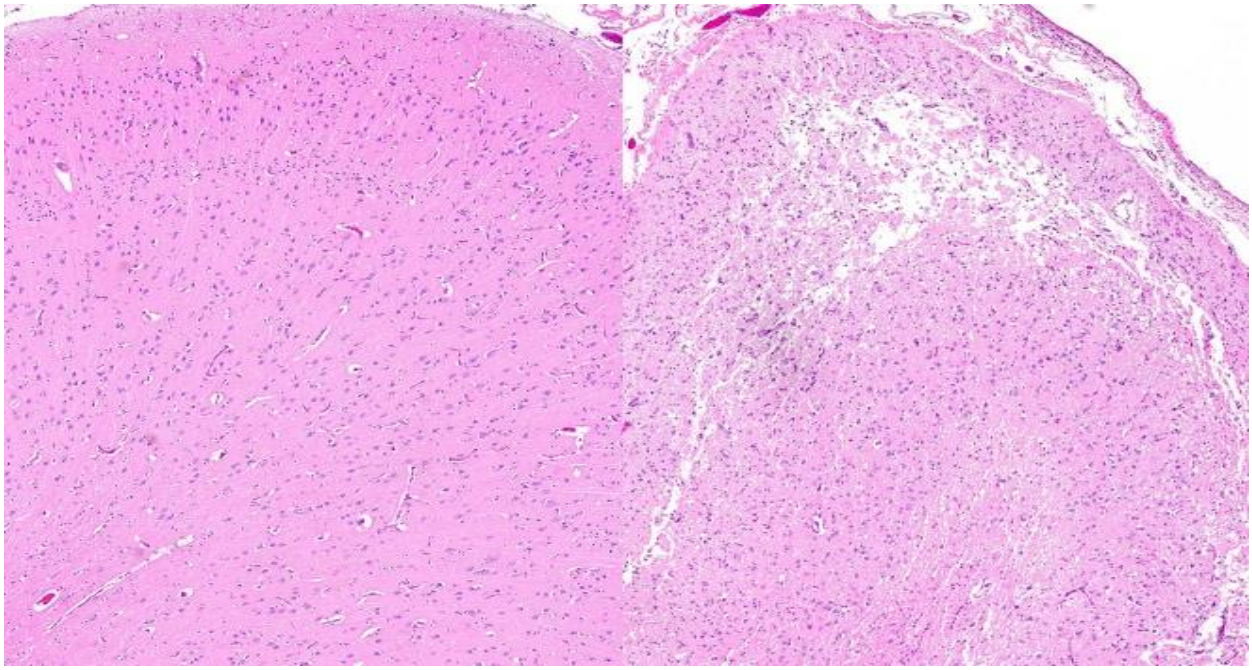


Cerebrum, goat: There is segmental laminar necrosis of the submeningeal gray matter (arrows) (Age and location-matched cerebrum at left.) (HE, 4X)

variable, with animals often presenting with facial twitching, teeth grinding, sal-ivation, blindness, seizures and opisthotonus.[8] The condition affects primarily young animals, and sheep and goats, as a rule, have a shorter course with fewer survivors. The syndrome is not always fatal; mortality rates are reported at 50-90%, although surviving animals typically have significant neurological deficits, including visual impairment and stupor.[4] Disease is seen worldwide and is responsible for important economic losses in many countries. The condition is more commonly seen in goats under intensive management conditions when fed more grain concentrate to encourage accelerated growth.[7]

PEM was recognized as a clinical and pathological entity long before specific pathogeneses had been discovered. Originally applied as a diagnosis to cattle and sheep losses in Colorado, the mor-phological designation of cerebrocortical malacia was subsequently used sy-nonymously for the specific entity of thiamine deficiency disease. However; it is now known that many cases of PEM in ruminants cannot be ascribed to thiamine deficiency.[2,3,6] There is often a lack of changes in thiamine concentration in ruminal fluid, tissue and blood in affected animals. Furthermore, there has been a failure to induce the disease by ex-perimentally created deficiencies. The most compelling argument for thiamine's role in PEM had been that administration of it in clinical cases, especially to those early in the disease course, often resulted in recovery. However, this is now believed to be related to improved energy metabolism in the impaired brain, regardless of the inciting cause.[5, 6, 7]

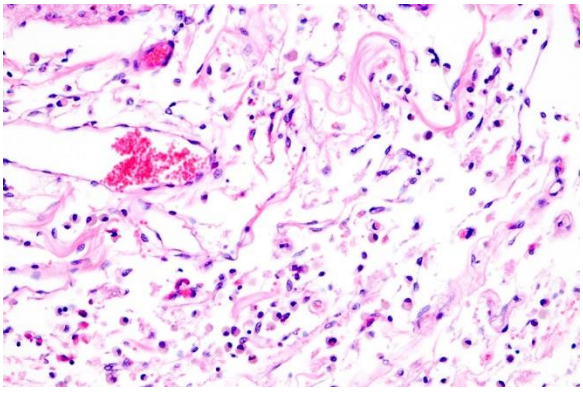
Currently, it is believed that PEM in ruminants can involve a wide range of pathogeneses, including toxic, metabolic, dietary/nutritional and even infectious events. In addition to thiamine deficiency, some of the specific causes of



Cerebrum, goat: Demonstration of laminar necrosis (right) in animal with PEM. (Age and location-matched cerebrum at left.) (HE, 40X) (Photo courtesy of: Division of Laboratory Animal Resources (DLAR) University of Pittsburgh, http://www.dlar.pitt.edu/

polioencephalomalacia in ruminants include sulfur poisoning, lead poisoning, salt poisoning (water deprivation), administration of levamisol or thiamine analogues such as amprolium, ingestion of thiaminase rich plants, and infection with bovine herpesvirus.[2]

Gross pathological changes are often striking, with the parietal-occipital cortex being most prominently affected. In acute cases, brains may have a swollen appearance and palpable softness, with flattening of gyri and narrowing of sulci. With more prolonged survival, as in this case, there is marked thinning or, in some areas, complete absence of friable, necrotic appearing grey matter, with zones of clefting/separation from underlying white matter visible.[8] The subarachnoid space is widened, and brains often appear smaller or shrunken. Areas of cerebrocortical necrosis (CCN) can be identified by autoflourescence under UV light, as a consequence of degraded lipoidal material within macrophages or high molecular weight collagen-like material. Although blood pyruvate levels may be elevated,[4] other serum biochemical analysis is variable and is generally of little value in disease diagnosis.



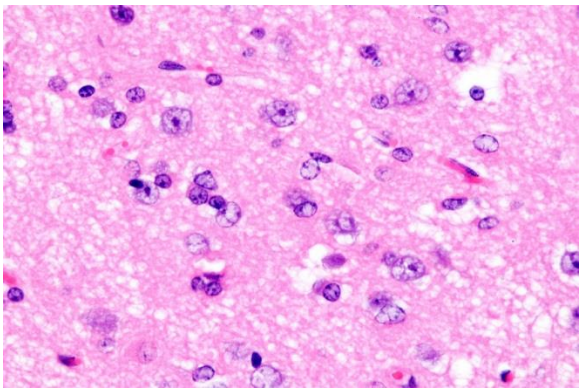
Cerebrum, goat: Remnant gliovascular strands in areas of laminar necrosis in a goat with PEM. (HE, 200X). (Photo courtesy of: Division of Laboratory Animal Resources (DLAR) University of Pittsburgh, http://www.dlar.pitt.edu/

As previously noted, the animal in question responded favorably to thiamine administration upon onset of signs, but continued to have significant neurological and visual deficits after stabilization.

JPC Diagnosis: Brain, cerebral cortex: Necrosis, laminar, multifocal to coalescing, with reactive gliosis.

Conference Comment: The contributor provides an excellent review of polioencephalomalacia, and the aged-matched control provided on the slide increases the teaching / learning value of this case. In ruminants, polioencephalomalacia is usually limited to the cerebrocortical grey matter and has a laminar pattern of distribution, often being referred to as "laminar cortical necrosis." The lesion in ruminants discussed above shares similarities with salt poisoning in swine and has been documented in cases of lead poisoning in cattle as mentioned in WSC Case 3 of this conference. It is most often a disease of young animals, although older animals may be affected sporadically.

Lesions of PEM vary in severity, depending on various factors such as species, age and duration.[1] Lesions are more severe grossly obvious in animals that survive for a period of time. The cerebral cortex often demonstrates superficial, laminar pallor which will trace the grey-white matter junction and may be most prominent in the gyri. Lesions are bilaterally symmetrical and are apparently more consistent in the caudal cerebral hemispheres. The distribution appears to be related to the area supplied by the middle cerebral artery.[1]



Cerebrum, goat: Adjacent to necrotic areas, small numbers of glial cells abut neurons. (HE, 400X). (Photo courtesy of: Division of Laboratory Animal Resources (DLAR) University of Pittsburgh, http://www.dlar.pitt.edu/

There is some slide variation in the severity of lesions in this case, but in general, it is representative of the classic microscopic lesions of PEM. Polioencephalomalacia does not have a specific etiology, as discussed above, but is often directly or indirectly linked to a deficiency in thiamine. Sulfur-containing compounds have also been implicated in some cases of PEM.[1] There is some question regarding the observed tissue autoflourescence in cases of PEM with some references stating it may originate from substances in mitochondria as opposed to ceroid-lipofuscin pigments.[10]

Contributing Institution:
Division of Laboratory Animal Resources (DLAR) University of Pittsburgh, http://www.dlar.pitt.edu/

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