Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2017-2018

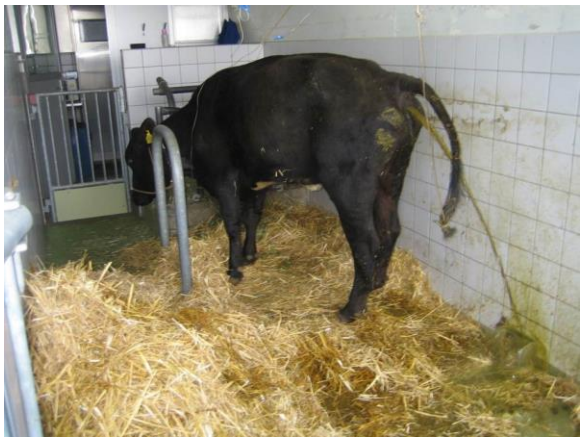
C o n f e r e n c e 10

29 November 2017

CASE I: H12/1754 (JPC 4019375).

Signalment: 3.5-year-old, Aberdeen angus, *Bos primigenius taurus*, bovine.

History: Out of a group of 6 animals, one cow showed chronic, profuse diarrhea and severe, progressive emaciation. The bacteriologic investigation of the feces tested positive for acid-fast rods and negative for Salmonella. Because of the suspicion of paratuberculosis, the cow was
euthanized and submitted for post-mortem investigation.



Presentation, ox. One of a group of 6 cattle displayed effortless and profuse diarrhea with concurrent emaciation. (Photo courtesy of: Institute of Animal Pathology, University of Berne, Länggassstrasse 122, Postfach 8466, CH-3001 Bern, Switzerland , http://www.itpa.vetsuisse.unibe.ch/htm)

Gross Pathology: The animal was moderately emaciated. The muscle masses were reduced, and the ribs were easily palpated. Body fat depots were present. The mucosa of the small intestine, from the duodenum to the ileum was moderate to severe thickened, corrugated, nodular shaped and were light brown. The large intestine content was watery and brown, becoming slightly mucoid in the rectum. No lesions in the colonic mucosa were noted grossly. The mesenteric lymph nodes were moderately enlarged. The rumen pH was 6.5, and the fibers of the content were up to 15 cm long.

Laboratory results:
Blood parameters:

Hematology: (changed parameters)

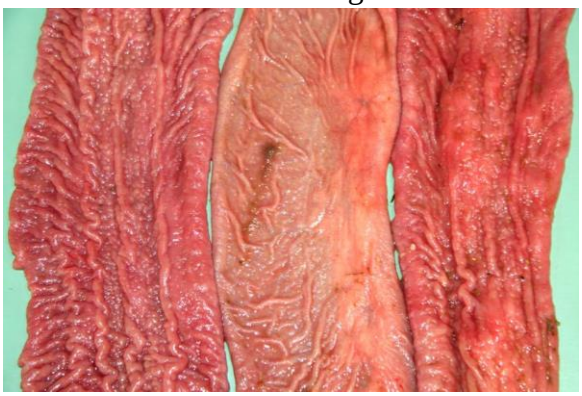
Banded Neutrophils	0.54	10^9/l	0	-	0.2
Segmented Neutrophils	6.25	10^9/l	1.0	-	3.5
Lymphocytes	2.05	10^9/l	2.5	-	5.5
Monocytes	0.93	10^9/l	0	-	0.33
Eosinophils	0.00	10^9/l	0.3	-	1.5

Chemistry: (changed parameters)

Na	116	mmol/l	135	-	165
K	1.50	mmol/l	3.0	-	6.0
Cl	68	mmol/l	90	-	110
Urea	23.99	mmol/l	1.67	-	7.50
Creatinine	183	µmol/l	88	-	133
Bilirubin	23.0	µmol/l	0.85	-	8.6
ASAT (SGOT)	79	IU	117	-	234
GGT	67	IU	10	-	27
GLDH	46	IU	0	-	17

Serology Bovine viral diarrhea (during Swiss eradication program): negative.

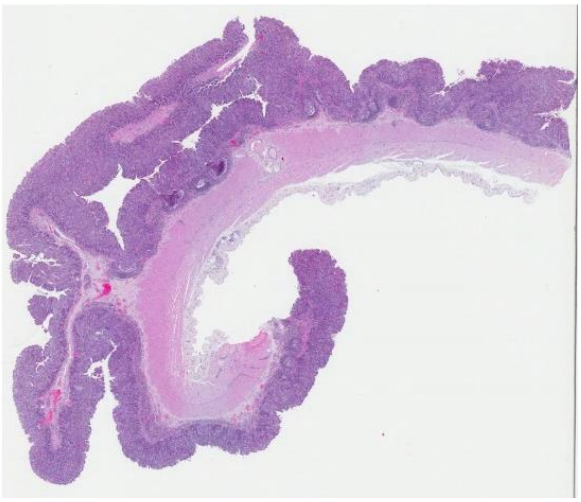
Microscopic Description: Small intestine: The intestine is thickened and puts the mucosa in folds. About 90% of the lamina propria and the submucosa are diffusely infiltrated by large numbers of epitheloid macrophages, lymphocytes, plasma cells, eosinophils and fewer neutrophils with numerous multinucleated giant cells of the Langhans and foreign body type. The infiltration distorts and expands the lamina propria of villi. Multifocally, the crypts are moderate to severe extended, lined by an elongated and flattened epithelium. They contain accumulations of cellular debris, mucous and crystalline material (dystrophic calcification) (cryptitis). Crypt epithelium piles up with cells 3-5 deep and with a high nuclear to cytoplasmic ratio (hyperplasia). The lamina propria, the submucosa and the serosa are diffusely widened and pale (edema). The submucosal and serosal lymphatics are diffusely moderately to severely dilated and surrounded by lymphocytes, plasma cells and fewer macrophages. Rarely epitheloid macrophages and multinucleated giant cells plug the lumen of the lymphatics (lymphangitis).



Intestine, ox. The intestinal mucosa is thickened, corrugated, and a segmentally light brown. (Photo courtesy of: Institute of Animal Pathology, University of Berne, Länggassstrasse 122, Postfach 8466, CH-3001 Bern, Switzerland, http://www.itpa.vetsuisse.unibe.ch/htm)

Contributor's Morphologic Diagnosis:
Small intestine: Granulomatous enteritis and lymphangitis with multinucleated giant cells, diffuse, severe, chronic.

Contributor's Comment: Lesions extended from the duodenum to the colon. During necropsy we were impressed about the classic lesion, as it is rarely seen in our necropsy room. The classic histologic picture likewise was a treat. Ziehl-Neelson stained (ZN) many acid-fast rods within the macrophages. The cow had a mild histiocytic lymphadenitis of the mesenteric lymph nodes with ZN-positive rods as well.



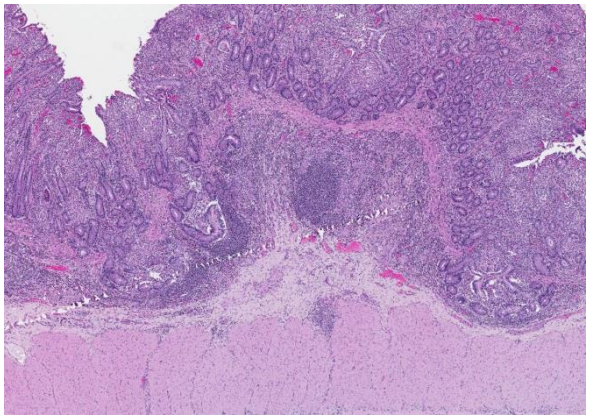
Intestine, ox. A section of thickened, hypercellular intestine is submitted for examination. (HE, 6X)

The diagnosis is paratuberculosis or Johne's disease. Although other infections can be seen on top of mycobacteriosis (e.g. salmonellosis), further bacteriologic investigation was not done.

Johne's disease (JD) or paratuberculosis, caused by *Mycobacterium (M.) avium* subsp. *paratuberculosis (Map)*, causes chronic diarrhea in ruminants. As in our case, typical gross lesions are thickened mucosa, thrown into transverse rugae which will not disappear when the intestinal tract is stretched. Typical microscopic lesions were granulomatous enteritis of the small intestine and lymphadenitis of the draining lymph nodes.

The bacteria are taken up orally by young animals. Susceptibility to infection is greatest in the first 30 days of life. The incubation period of JD is protracted and clinical symptoms are usually detected in cattle 2-5 years-old. Chronic villous involvement leads to malabsorption, protein loss and profuse chronic diarrhea and severe emaciation. The pathogenesis of JD is best understood in cattle. It is assumed to be similar in other ruminants, except that in sheep and goats the enteric gross lesions are often milder. *Map* can be produced in pigs. Spontaneous disease occurs in a number of free-ranging and captive wild ruminants, camelids, rarely in equines and captive primates. Numerous species of wild mammals and several species of wild birds are naturally infected, though not necessarily diseased.[1]

Map has been suspected to play a role in Crohn's disease (CD), a chronic inflammatory bowel disease in humans. Initial suggestion of Map involvement was based on the similarity of the clinical appearance of CD and JD. *Map* has been detected in multiple CD studies, but it is difficult to isolate *Map* from patients with CD. Genetically, over 30 Map genes have been identified in human disease, but no specific association has been shown so far.

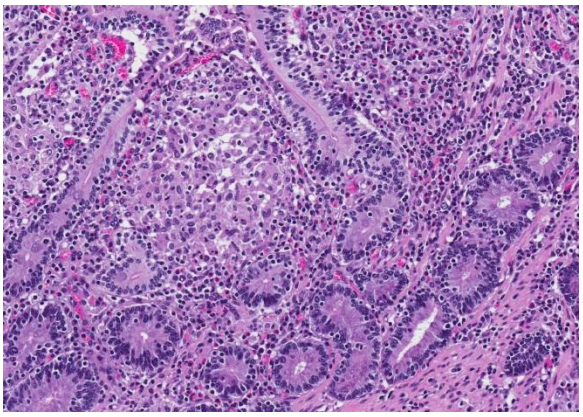


Intestine, ox. There is overall thickening of the intestinal mucosa with loss of villi and crypts, which are replaced by a dense cellular infiltrate. A few remaining hyperplastic crypts extend down into underlying Peyer's patches. (HE 35X)

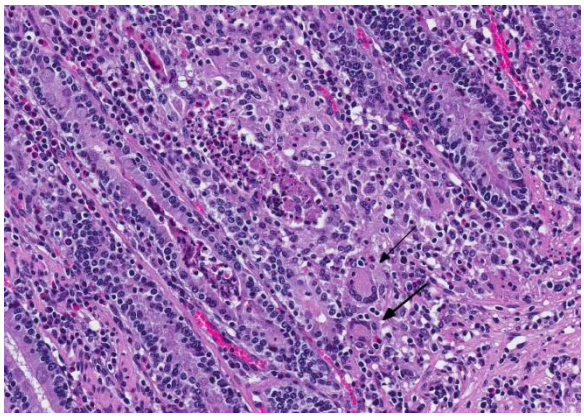
JD is used as a bovine model of CD. Studies in cattle suggest the early immune response may be similar to the immune response to *M. tuberculosis (Mtb)* during the latent stage of infection. *Map* affords an opportunity to examine the immune response during the early and late stages of infection. These data would also provide insight into how mycobacterial pathogens could contribute to the pathogenesis of CD.[2]

JPC Diagnosis: Small intestine: Enteritis, granulomatous and lymphocytic, diffuse, marked with villar blunting, crypt abscessation and loss, and moderate lymphangitis, Aberdeen angus (*Bos primigenius Taurus)*, bovine.

Conference Comment: Johne's disease in cattle, sheep, and goats is caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and induces granulomatous inflammation of the lepromatous (diffuse) type. The immune response is characterized by a Th2 type of adaptive immune response and appears microscopically as diffuse sheets of macrophages and multinucleated giant cells rather than distinct granulomas as would be expected with a Th1 response.



Intestine, ox. The cellular infiltrate extends into the underlying submucosa and tracks vessels. The infiltrate which replaces crypts is composed of numerous polygonal epithelioid macrophage admixed with fewer neutrophils. (HE, 1 56X)



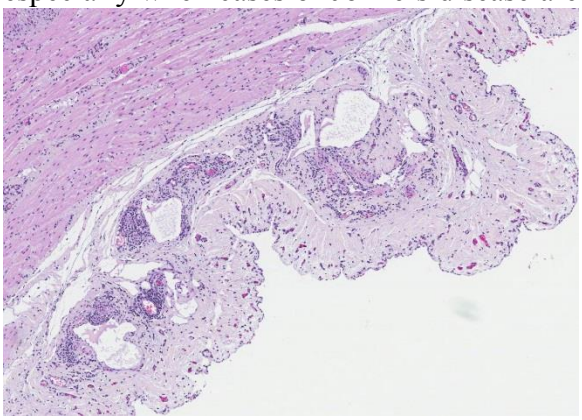
Intestine, ox. Two Langhans-type giant cell macrophages (arrows) are present within the histiocytic infiltrate adjacent to two crypt abscesses. (HE, 228X)

Lesions are most common in the ileum, colon, and mesenteric lymph nodes. Bacteria, which are often numerous, can be identified within macrophages and extracellularly with acid-fast stains. Johne's disease causes injury to cells in three ways: (1) lysis of epithelial cells and extracellular matrix proteins that form cell junctional barriers in the small intestinal mucosa, (2) dysfunction of afferent lymphatic drainage in the small intestinal villi, and (3) lysis of monocyte-macrophage cells and other cells within the lamina propria of infected intestinal villi from chronic inflammatory mediators.[3]

Grossly, affected small intestinal walls are thickened with a cerebriform appearance and mesenteric lymph nodes are enlarged with coalescing areas of yellow-white caseous exudate which occasionally mineralizes. Lymphangitis is common resulting in thickened cords of lymphatic vessels coursing through the mesentery. Additionally, there is marked muscle loss and wasting with intermandibular edema (attributable to hypoproteinemia), fluid accumulation in body cavities, plaques of mineralization and fibrosis within the tunica intima of the thoracic aorta, and diffuse, watery diarrhea. Young animals are most susceptible, and are infected through

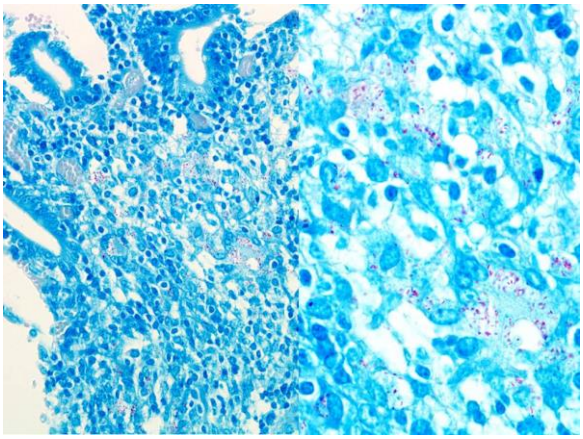
ingestion of the bacterium which binds to receptors on the luminal surfaces of M (microfold) cells (which lack a mucous covering). Bacteria are then translocated across the cell into the underlying Peyer's patches and subsequently phagocytosed by tissue macrophages. MAP requires iron for growth and secretes iron-chelating proteins known as exochelins, iron-reductases, and siderophores as virulence factors to acquire iron from ferritin stored in macrophages. Additionally, mycobacterium species can: (1) inhibit acidification of the phagosome, fusion of the phagosome and lysosome, and lysosomal enzyme activities through the production of peroxidases; (2) block injury from reactive oxygen and nitrogen intermediates; and (3) suppress macrophage activation by cytokines (IFN-γ).[3]

There was apathetic debate amongst conference attendees regarding the use of granulomatous versus lymphoplasmacytic versus histiocytic to describe the inflammatory infiltrate in this case, a debate which has been oft-repeated over the years, especially when cases of Johne's disease are



Intestine, ox. Dilated lymphatics within the edematous serosa are surrounded by low to moderate numbers of lymphocytes and fewer histiocytes and plasma cells (lymphangitis). (HE, 80X)

discussed. In this particular case, the presence of multinucleated giant cells and epithelioid macrophages suggest a granulomatous process, their presence



Intestine ox: Mucosal histiocytes contain moderate numbers of acid fast bacilli consistent with M. paratuberculosis. (Ziehl-Nielsen, 400X)

however, was restricted to the submucosa and further out, especially around lymphatics. Lymphocytes, however, predominate in the lesion. Ultimately, the group decided that the presence of numerous epithelioid macrophages in the mucosa as well as the multinucleated macrophages warranted the use of granulomatous in this particular instance.

Contributing Institution:

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http://www.itpa.vetsuisse.unibe.ch/htm

References:

1. Brown CC, Baker DC, Barker IK. Alimentary system. In Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 5th ed. Vol. 2. Philadelphia, PA: Elsevier; 2007:222-225.

2. Davis WC, Madsen-Bouterse SA. Crohn's disease and *Mycobacterium avium* subsp. *paratatuberculosis*: The need for a study is long overdue. *Vet. Immunol. Immpathol*. 2012;145:1-6.

3. Zachary JF. Mechanisms of microbial infections. In: Zachary JF, ed.

Pathologic Basis of Veterinary Disease. 6th ed. St. Louis, MO: Elsevier; 2017:162-163.

CASE II: JCP-TAMU-1 2017 (JPC 4102431).

Signalment: 1.5-year-old, Domestic shorthair, *Felis catus*, feline.

History: This 1.5-year-old, castrated-male DSH (*Felis catus*) was taken to the rDVM on a Tuesday for anorexia, vomiting, and not being as "vocal" as normal. He was treated with an anti-emetic (maropitant and cerenia) and sent home. He hid under the bed all day and had a "seizure." The cat returned to the rDVM on Wednesday where he had a seizure and was hypersalivating. The RDVM did a serum chemistry diagnosing renal failure (Creatinine >20mg/dL (0.3-2.1 mg/dL) and BUN>200 mg/dL (<35 mg/dL)). The cat had elevated serum amylase (2263 U/L (300-1100 U/L)). The cat was referred to TAMU emergency receiving. The cat was dehydrated and quiet but otherwise normal physically. The owner was worried that the cat ate some lilies she had received in Monday. Bloodwork showed severe renal failure. A poor prognosis was given and euthanasia was elected late Tuesday night.

Kidney, cat. The kidney was unremarkable. (Photo courtesy of: Texas A&M University, College of Veterinary Medicine and Biomedical Sciences, (Departmental Web site address): http://vetmed.yamu.edu/vtpb)

Gross Pathology: A 4.9 kg (10.8 lb), 1.5-year-old, castrated-male, orange tabby Domestic Shorthair cat in good body condition is autopsied on January 19, 2017.

INTEGUMENTARY/SPECIAL SENSES: A 4 cm circumferential band on the left antebrachium and 2x2 cm on the plantar surface of the left crus are shaved. A moderate amount of brown debris (cerumen) is in both external ear canals.

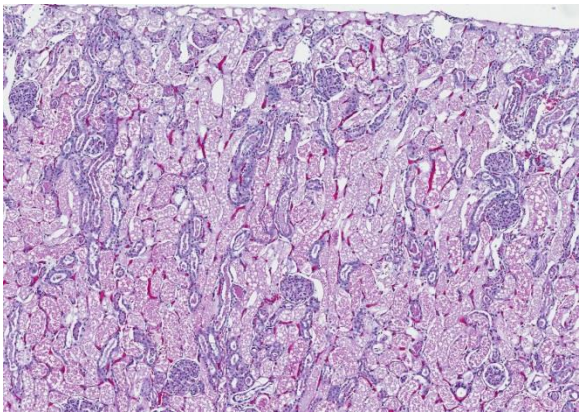
RESPIRATORY: The lungs are diffusely dark red, glistening, and wet, and on section, ooze abundant, red, foamy fluid into the airways (pulmonary edema). A 1.5x2 cm, pale pink, hyperinflated area is at the margins of the cranial lung lobes (emphysema).

CARDIOVASCULAR (Heart weight: 26 g; Right ventricular wall: 1.5 mm; Left ventricular wall: 4 mm)**, MUSCULOSKELETAL**, **HEMIC & LYMPHATIC** (Spleen weight: 20 g), **ENDOCRINE**, **URINARY** (Right kidney weight: 20 g; Left kidney weight: 20 g), **GENITAL**, **DIGESTIVE**, **LIVER/PANCREAS** (Liver weight: 106 g), **NERVOUS** (Brain weight: 28 g): No significant findings.

Laboratory results:

Blood pressure 120 mmHg (120-180 mmHg)

Severe acidosis pH=7.158 (7.38-7.49))



Kidney, cortex. There is extensive necrosis of proximal convoluted tubules and filling of the lumen with light pink proteinaceous and cellular debris. Regenerating tubular epithelium is deeply basophilic. (HE, 50X)

Hypermagnesimic 0.86 mmol/L (0.38-0.52 mmol/L)
Moderate hyperkalemia 5.09 mmol/L (3.91-4.4 mmol/L),
Azotemia = elevated BUN (250 mg/dL (7-32 mg/dL)) and creatinine 12 mg/dL (0.6-1.9 mg/dL))
Hyperosmotic = osmolarity 377.8 mOsm/kg (291-309 mOsm/kg)

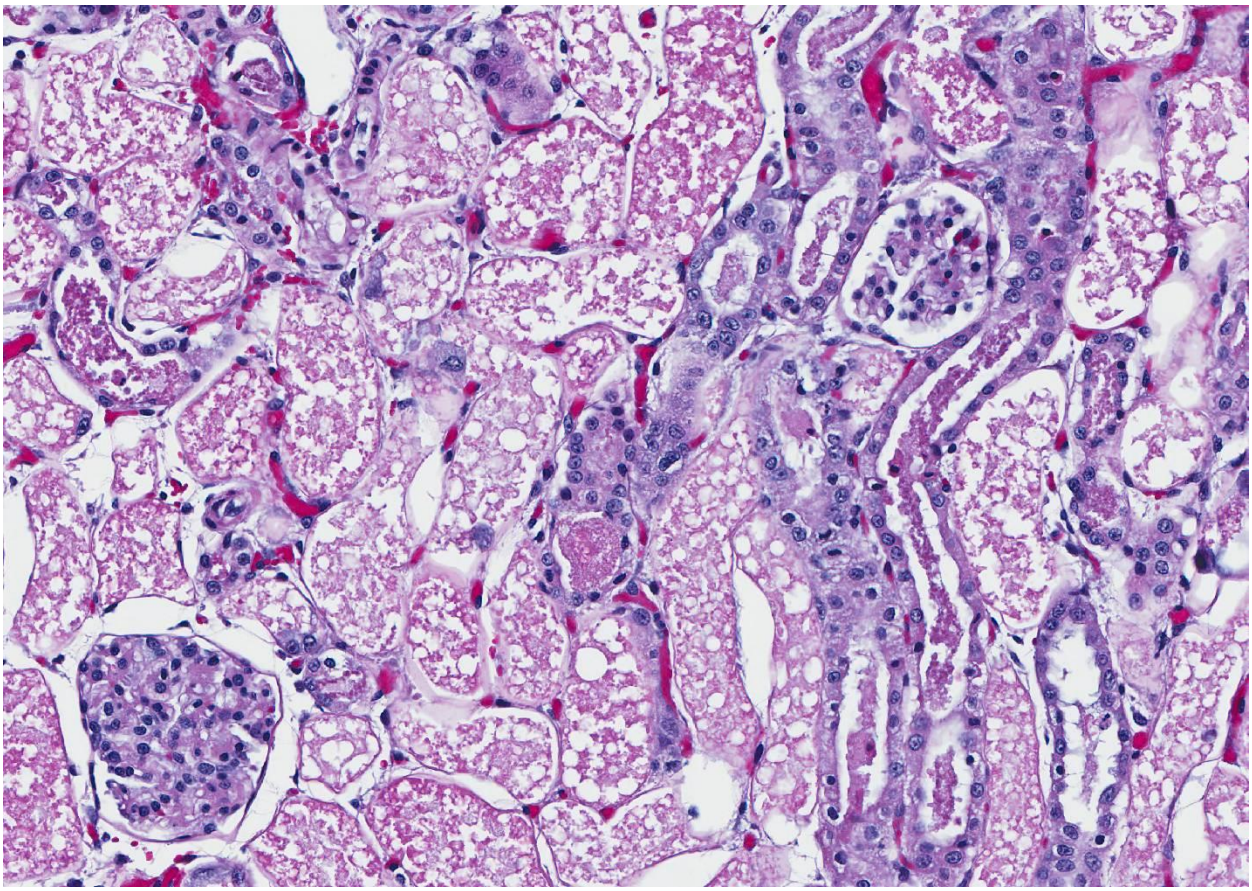
Kidney scraping at necropsy: few birefringent (oxalate presumed) crystals (physiologic normal)

Macroscopically the kidneys were unremarkable.

Microscopic Description: Kidney: The section of kidney contains the cortical surface through to the collecting tubules. The proximal tubules are uniformly affected with necrosis and sloughing of tubular cells to fill their lumens. The denuded basement membranes remain intact. Glomeruli are unaffected, and the interstitium is expanded in patches of tubules and around large vessels. Moving toward the cortico-medulary junction, tubules begin to have a granular content, and surviving, attenuated tubular cells surround granular cell debris (granular casts). The regenerating tubular cells often have karyomegaly, and the lining cells progressively become more numerous in tubular profiles. Occasionally, deep blue homogeneous orbs are in cell debris (presumed nucleic acid cohesions). In the medullary tubules and collecting ducts the lining cells are normal and tubules contain occasional granular casts. The medulla has a few foci of nephrocalcinosis. Few, presumed, pre-existing foci of interstitial mononuclear inflammation are noted**.**

Contributor's Morphologic Diagnosis:
Kidney: Severe subacute proximal tubular necrosis/nephrosis, severe subacute proximal tubular injury; granular tubular casts; tubular regeneration.

Contributor's Comment: This is a straight forward case of lily intoxication in a cat. [1-3, 6-7, 9] Early onset of vomition, anorexia, hypersalivation, and apathy with rapid progression and later, seizures is usually reported. Unfortunately, as in this case, if early, immediate, rigorous gastric cleansing and fluid diuresis are not instituted, these cases are fatal. The renal lesion is acute proximal tubular necrosis with rupture and sloughing of lining cells leaving tubules full of indistinct cell debris. Ultrastructurally, the cells are characterized by early (<8 hours post exposure) disruption of apical crista, nuclear pyknosis, megamitochondria and some lipid droplet formation.[8] The lesions are thought to be the result of hypoxia related to mitochondrial intoxication, and the megamitochondria are the product of mitochondrial fusion. The actual toxins are thought to be several steroidal glycoalkaloids, but are undefined.[10] Aqueous extracts of all parts are toxic, and flowers are believed to be more toxic than leaves. Actual experimental cat experiments are limited, but the toxicity curve is steep and different fractions of aqueous extracts, though toxic, are of variable toxicity.



Kidney, cortex. Higher magnification of the diffuse tubular epithelial necrosis. Approximately 15% of the epithelium has begun to regenerate. Glomeruli are within normal limits. (HE, 176X)

Another lesion seen in cats is pancreatic acinar cell degeneration. [3, 8] It is a subtle lesion consisting of numerous, small vacuoles especially prominent in the basophilic cytoplasmic zone of acinar cells which was seen in this cat. Ultra-structurally, these vacuoles presumably represent lipid vacuoles, again, the result of hypoxia. The increase in amylase reported in some cases, including the one presented, is thought to reflect the acinar cell damage.

Seizuring is reported terminally as in this cat. Although thought to be the result of uremia, the cause of seizuring is felt by some to be more complicated and perhaps involves the effect of the toxin because these seizures are triggered by handling.[8]

Exposure to lilies inevitably occurs in households with cats that have lilies of many species (growing or delivered as gifts) because cats will seek them out.[9] A tragic but fascinating condition.

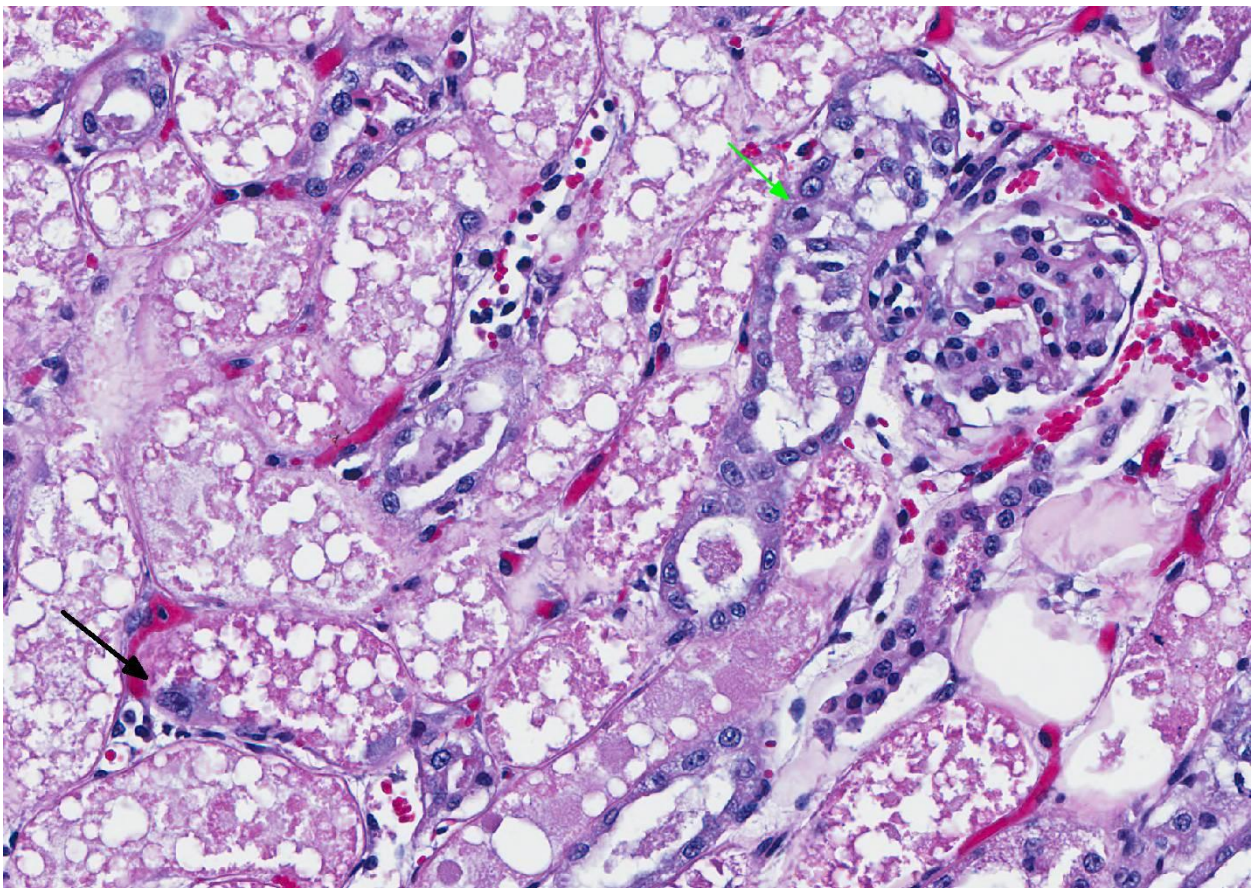
JPC Diagnosis: Kidney, proximal convoluted tubules: Necrosis, diffuse with regeneration and granular cast formation, Domestic shorthair (*Felis catus*), feline.

Conference Comment: Several members of the *Liliaceae* family affect domestic animals, namely, *Lilium* spp. in cats and *Narthecium ossifragum* (bog asphodel) in ruminants. Cats and ruminants are affected by ingestion of leaves or flowers of the plants and present with acute onset polyuria, polydipsia, glucouria, proteinuria,

isosthenuria, and azotemia.[5] The toxic ingredient is unknown, but the acute proximal tubular necrosis that occurs in the kidneys is most likely a result of hypoxia. The most common genera of lily plants, the Easter lily (Lilium longiflorum), affects cats seasonally when plants are brought into their environment; other species: day lily (Hemerocallis spp.), tiger lily (Lilium sp.), Japanese show lily (Lilium hybridum), and rubrum lily (Lilium rubrum) also cause identical renal lesions in cats.[4] Additionally, cats have acute pancreatic necrosis and elevated creatinine kinase. Renal tubular epithelial cells have swollen mitochondria, megamitochondria, and accumulation of lipid droplets ultrastructurally.[5]

The presence of intact basement membranes was demonstrated in the conference with a periodic acid-Schiff with methenamine (PAMS) stain. This stain may be useful in demonstrating tubulorrhexis (induced by ischemia), or its absence (suggestive of acute toxicities). Other differentials for this particular lesion which were briefly discussed included heavy metals, acetaminophen, trimethylprim sulfa, bee or snake venom, various chemotherapeutics, and ethylene glycol. Ethylene glycol could be immediately ruled out in this case because of a lack of intratubular crystal formation.

The blue "orbs" mentioned by the contributor were noticed and admired by conference participants. We agree that they are most likely nucleic acid deposition and are reminiscent of nuclear debris seen in acute tumor lysis syndrome, although their



Kidney, cortex. Regenerating epithelium displays mitotic figures (green arrow) and karyomegaly (black arrow). (HE, 202X)

presence in renal tubules is unique.

Contributing Institution:
Texas A&M University
College of Veterinary Medicine and Biomedical Sciences
http://vetmed.tamu.edu/vtpb

References:

1. Bennett AJ, Reineke EL. Outcome following gastrointestinal tract decontamination and intravenous fluid diuresis in cats with known lily ingestion: 25 cases (2001–2010). *J Am Vet Med Assoc.* 2013;242:1110–1116.
2. Berg RIM, Francey T, Segev G. Resolution of acute kidney injury in a cat after lily (*Lilium lancifolium*) intoxication. *J Vet Intern Med*. 2007;21:857–859.
3. Brady MA, Janovitz EB. Nephrotoxicosis in a cat following ingestion of Asiatic hybrid lily (*Lilium* sp.). *J Vet Diagn Invest*. 2000;12:566–568.
4. Breshears MA, Confer AW. The urinary system. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease.* 6th ed. St. Louis, MO: Elsevier; 2017:681.
5. Cianciolo RE, Mohr FC. Urinary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals.* Vol. 2. 6th ed. St. Louis, MO: Elsevier; 2016:428.
6. Fitzgerald KT. Lily toxicity in the cat. *Topics Comp Anim Med.* 2010;25:213-217.
7. Langston CE. Acute renal failure caused by lily ingestion in six cats. *J Am Vet Med Assoc*. 2002;220:49–52.
8. Rumbeiha WK, Francis JA, Scott D, et al. A comprehensive study of Easter lily poisoning in cats. *J Vet Diagn Invest*. 2004;16:527–541.
9. Slater MR, Gwaltney-Brant S. Exposure circumstances and outcomes of 48 households with 57 cats exposed to toxic lily species. *J Am Anim Hosp Assoc*. 2011;47:386–390.
10. Uhlig S, Hussain F, Wisløff H. Bioassay-guided fractionation of extracts from Easter lily (*Lilium longiflorum*) flowers reveals unprecedented structural variability of steroidal glycoalkaloids. *Toxicon.* 2012;92:42-49.

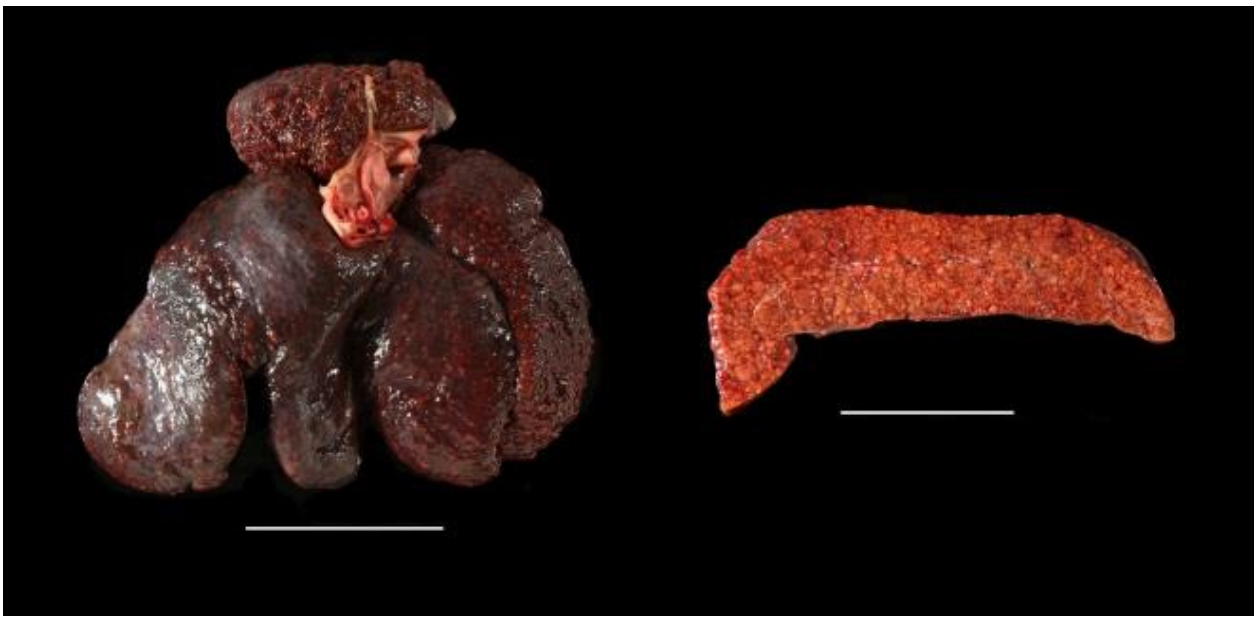
CASE III: N-103/17 (JPC 4102670).

Signalment: 7-year-old, male, neutered, Doberman pinscher, *Canis familiaris*, canine.

History: This 7-year-old Doberman was presented with recurrent peritoneal and pleural effusions of 3 weeks duration. Echocardiography was performed without heart alterations. Blood biochemical examination revealed increased hepatic enzymes and decreased albumin parameters. A marked lack of coagulation factors was also detected without improvement after plasma transfusions. No more clinical information was supplied.

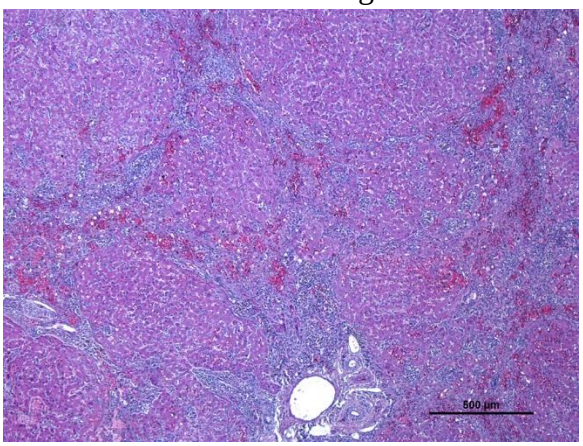
Gross Pathology: Mucous membranes were icteric and the abdomen was visibly distended. Approximately 9 litres of clear yellowish free fluid in the abdominal cavity were observed, and 3 litres of similar fluid was present in the thoracic cavity. The liver was diffusely yellow to tan, decreased in size and showed multiple multifocal to coalescing micro-nodulations.

Laboratory results:
None provided.

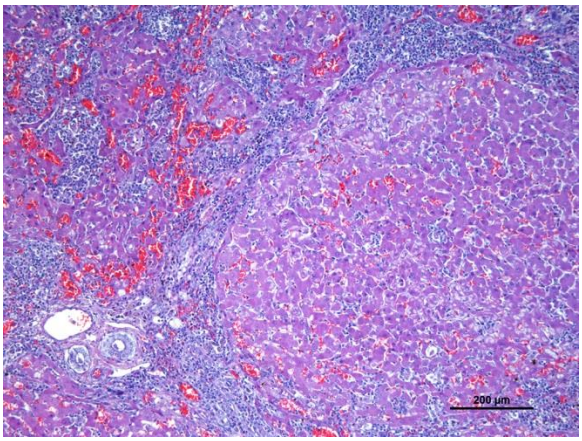


Liver, dog. The liver is small with numerous regenerative nodules. On cut section, the liver is yellow-tan, and nodular regeneration is even more obvious . (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

Microscopic Description: Liver: Diffusely affecting all the section there is a severe chronic degenerative process together with multifocal nodules of hepatic regeneration. There is a markedly reduction of the diameter of hepatic lobules because of loss of numerous hepatocytes. Marked portal-to-portal bridging is observed. Which is composed of moderate biliary hyperplasia and mild fibrosis along with severe

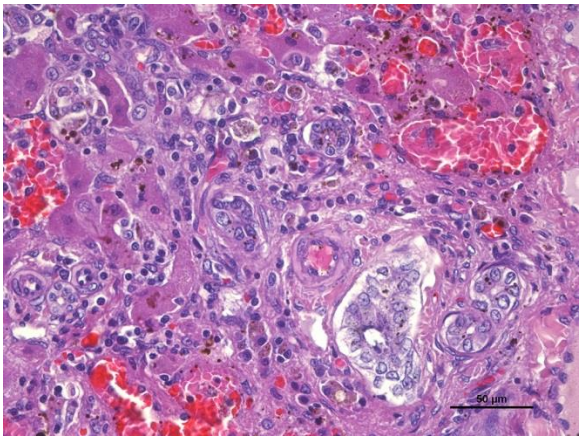


Liver, dog. There is marked periloabular fibrosis and a reduction in the size of the lobules with loss of periportal hepatocytes and edema. (HE, 40X) (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)



Liver, dog. Higher magnification of a regenerative nodule with periportal fibrosis and inflammation. (HE, 100X) (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

inflammatory infiltrate mainly compose of macrophages (most of them arranged in aggregates) and abundant lympho-plasmacytic cells. Numerous biliary ducts contain moderate amount of amorphous yellowish material in the lumen and small bile casts are located in canaliculi (cholestasis). Biliary pigment is also located



Liver, dog. Portal areas contain numerous biliary profiles as well as individual and aggregates of macrophages with brown intracytoplasmic pigment. Fibrosis surrounds and entraps periportal hepatocytes, with also contain brown granular pigment, and sinusoids are multifocally dilated. (HE, 400X) (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

in the cytoplasm of Kupffer cells and hepatocytes. Multifocally sinusoids are distorted, dilated and markedly congested. Numerous hepatocytes appear enlarged with marked eosinophilic nucleolus. Degenerated changes of centrilobular hepatocytes can be seen: intracytoplasmic non-stained, well-defined micro vacuoles (lipid vacuoles) and cytoplasm rarefaction (hydropic degeneration). Also, scattered hepatocytes appear individualised, hypereosinophilic and karyorrhectic (apoptotic hepatocytes). There are multifocal areas of hepatic regeneration consistent of well-delimitated nodules of viable hepatocytes surrounded by fibrous tissue. Cells of the hepatic capsule appear plump and reactive.

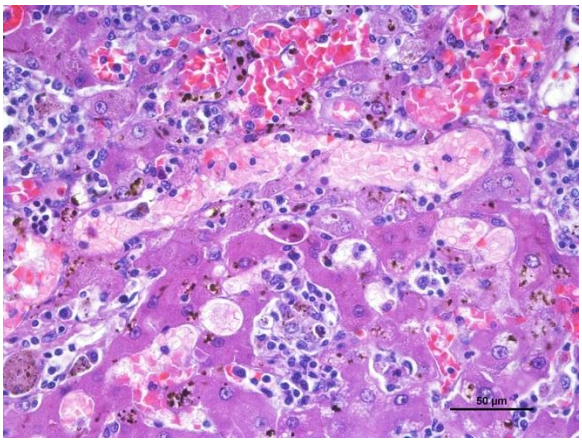
SPECIAL STAINS: Rubeanic acid stain was performed and abundant green granular pigment observed in the cytoplasm of numerous hepatocytes in periportal areas.

Contributor's Morphologic Diagnosis:
Liver: Chronic, diffuse, severe hepatocellular degeneration and loss, with intrahepatocellular copper, severe bile stasis, nodular regeneration and moderate lipidosis (end-stage liver (cirrhosis)), Doberman, canine.

Contributor's Comment: The alteration of clinical parameters observed in this case (hypoalbuminemia, hepatic enzymes increased and lack of coagulation factors) was secondary to chronic hepatic failure. Hypoalbuminemia lead to multiple effusions in body cavities and jaundice was secondary to a severe biliary stasis.

In this case, acid rubeanic tissue staining revealed numerous intracytoplasmic green granules in hepatocytes. Therefore, the primary cause of the chronic hepatic failure and the end-stage liver was the chronic copper accumulation and toxicity.

Hepatic injury in copper poisoning of domestic animals frequently is the result of progressive accumulation of copper within



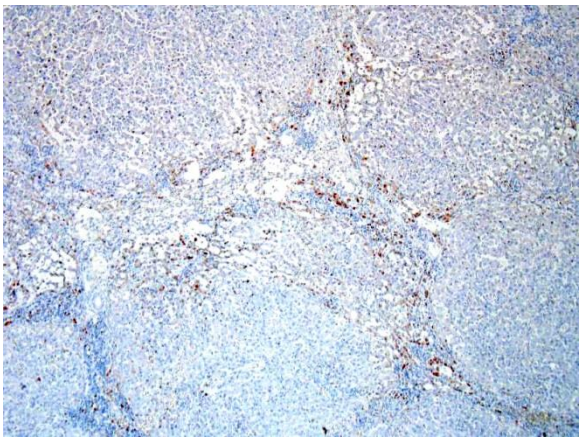
Liver, dog. Occasional hepatocytes (center) are disassociated, hypereosinophilic, and shrunken (apoptosis). (HE, 400X) (Photo courtesy of: Veterinary Pathology Department, Veterinary Faculty, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain.)

the liver. Hepatic copper toxicosis can result from a primary metabolic defect in hepatic copper metabolism, altered hepatic biliary excretion of copper, or from excess dietary intake of the element.[2]

Hepatic copper accumulation in association with chronic hepatitis has been documented in Doberman, but apart from the recognized genetic mutation in the COMMD1 gene in Bedlington Terriers, leading to a primary copper storage disease with impaired copper excretion, the pathogenesis of the Doberman copper accumulation remains unclear.[2,4]

Copper is an essential trace element of all cells, but even a modest excess of copper can be life-threatening because copper must be properly sequestered to prevent toxicosis. Normally, serum copper is bound to ceruloplasmin and the majority of hepatic copper is bound to metallothionein and stored in lysosomes. Excess copper can lead to the production of reactive oxygen species that initiate destructive lipid peroxidation reactions that affect the mitochondria and other cellular membranes.[1]

In cases of chronic hepatitis caused by copper accumulation, the liver is usually small, often with an accentuated lobular pattern; severely affected livers are characterized by architectural distortion, which ranges from a coarsely nodular texture to an end-stage liver. Chronic hepatitis, depending on the duration of inflammation and injury, is characterized by portal and periportal mononuclear cell inflammation and fibrosis of portal areas that may extend into adjacent periportal areas of the lobule, leading to the prominent lobular pattern. Small aggregates of pigmented macrophages, containing copper and lipofuscin, surrounded by mononuclear inflammatory cells are a reliable feature of copper excess. With progression, hyperplastic nodules and bridging fibrosis develop.[3]

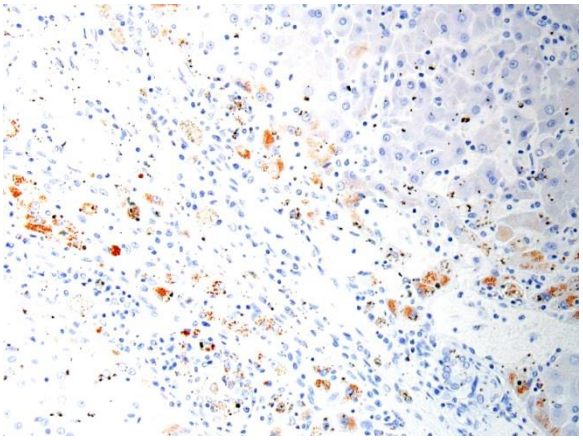


Liver, dog. Low magnification view of copper-laden hepatocytes within hepatic parenchyma separating regenerative nodules. (Rhodanine, 100X)

JPC Diagnosis: Liver: Fibrosis, bridging and portal, diffuse, severe with macronodular hepatocellular regeneration, piecemeal necrosis, cholestasis, siderosis, and sinusoidal capillarization, Doberman pinscher (*Canis familiaris*), canine.

Conference Comment: Doberman Pinchers are one of a number of dog breeds (Bedlington Terrier, West Highland White Terrier, Labrador Retriever, American and English Cocker Spaniel, Skye Terrier, Standard Poodle, Dalmatian, and English Springer Spaniel) that are predisposed to chronic hepatitis. In some of the aforementioned breeds genetic mutations have been identified (Bedlington Terriers with mutations in the COMMD1 gene are described above) but the majority of them are idiopathic. There is an association between excess copper accumulation and chronic liver disease but the exact mechanism has not been worked out yet.[2] Sheep are especially sensitive to excess copper because they don't efficiently regulate copper storage. Although copper is a necessary element for cellular metabolism,

it is far from innocuous, and must be sequestered to prevent toxicosis. In serum, it is bound to ceruloplasmin, and in the liver, it is bound to metallothionein and stored in lysosomes. Even small amounts of unbound, excess copper, results in the production of reactive oxygen species that lead to lipid peroxidation causing mitochondrial and cell membrane damage. Copper toxicosis can occur by the following mechanisms: (1) dietary excess (especially in sheep); (2) inadequate molybdenum in feed which normally antagonizes copper; (3) ingestion of pyrrolizidine alkaloids (*Heliotropium, Crotalaria, Senecio* species) which prevent hepatocyte mitosis which increases copper load on surviving hepatocytes; (4) disorders in copper metabolism (mentioned above).[1]



Liver, dog. Higher magnification of copper-laden hepatocytes. (Rhodanine, 200X)

This case engendered spirited discussion concerning the location of the copper and its causality of the chronic changes in this animal. In this individual, the copper is located exclusively in periportal regions, as is seen in many cases of chronic hepatitis. The majority of the parenchyma that is replaced by nodules of regenerating hepatocytes has not accumulated copper; relegating the demonstration of copper to the small amount of "original" liver present in the slide. Whether regenerative nodules do not accumulate copper as a result of an adaptive response or simply as a result of their relatively recent maturation, has not been conclusively determined.[2] However, at this point in lesion development, the attendees believe that it is difficult, if not impossible to determine the link between the copper accumulation in hepatocytes and its causality, if any, to the ongoing cirrhotic process.

Another very interesting change in this liver is the dilation of periportal sinusoids, which resembles "sinusoidal capillarization", a change described in chronic hepatitis in humans.

In the normal animal, liver sinusoidal endothelium varies markedly from capillary endothelium in other organs, as it exhibits fenestration, lacks a basement membrane, and does not express factor VIII-related antigen , platelet endothelial cell adhesion molecules (PECAM-1), CD-34, or E-selectin. In addition, affected sinusoids exhibit decreased compliance with sinusoidal blood flow (likely resulting in the dilation noted in this slide in periportal sinusoids) and possibly contributing to portal hypertension.[4]

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References:

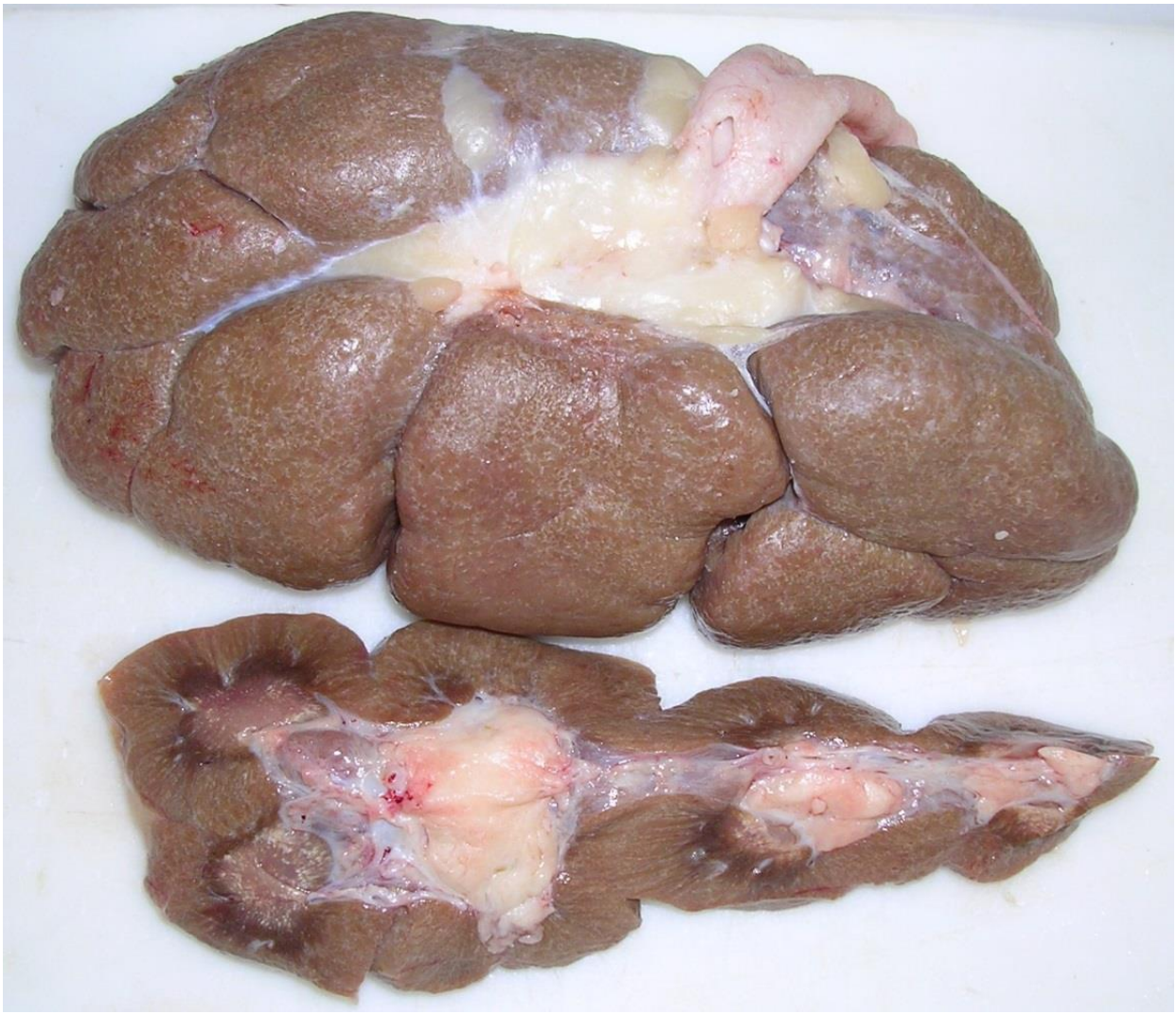
1. Brown DL, Van Wettere AJ, Cullen JM. Hepatobiliary system and exocrine pancreas. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 6th ed. St Louis, MO: Elsevier Mosby; 2017: 440.
2. Cullen JM, Stalker MJ. Liver and biliary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. Vol. 2. 6th ed. St

Louis, MO: Elsevier Mosby; 2016: 302-303, 342.
3. Mandigers PJ, Van den Ingh TS, Spee B, Penning LC, Bode P, Rothuizen J. Chronic hepatitis in doberman pinschers: a review. *Vet Q*. 2004;26(3):98-106.
4. Xu B, Broome U, Uzumel S, Ge, X, Kumaga-Baaesch M, Huttenby K, Christenson B, Ericzon B, Holgersson J, Sumitran-Holgersson S. Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. *Am J Pathol* 2003 163(4) 1275-1289.

CASE IV: 2014 Case 1 (JPC 4050142).

Signalment: Adult, female, breed unspecified, *Bos taurus*, bovine.

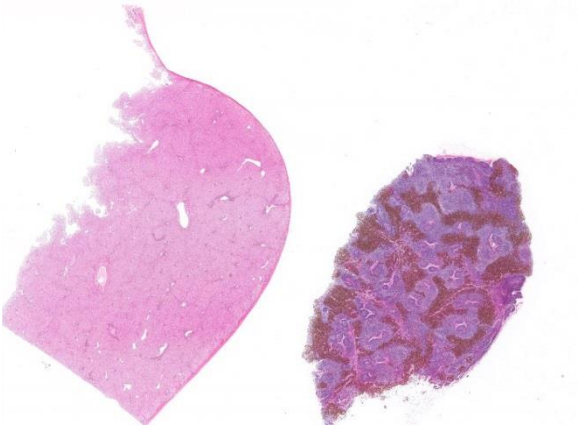
History: The bovine was presented for slaughter at a USDA Food Safety Inspection Service (FSIS) inspected slaughter facility and passed antemortem inspection. The carcass was retained for further diagnostic testing after green, caseous-appearing lesions were observed in the liver and portal lymph nodes during postmortem inspection.



Kidney, ox. Greenish discoloration of the renal cortex of an ox at slaughter. (HE, 5X)

Gross Pathology: Green, caseous-appearing lesions in the liver and portal lymph nodes.

Laboratory results:
None provided.



Lymph node and kidney. The medullary sinuses of the lymph node contains abundant brown granular pigment; small aggregates of pigment are present within the renal cortex. (HE, 6X)

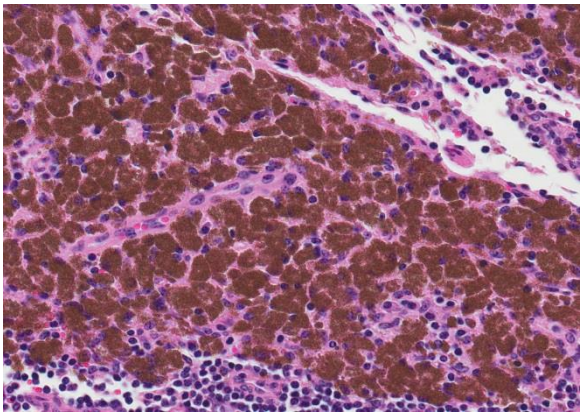
Microscopic Description: Liver: Predominantly in portal areas, macrophages contain intracytoplasmic light green to light tan crystalline material which is birefringent when viewed under polarized light. Individual crystals are irregular to rod-shaped and range in size from <1µm to approximately 3µm in length and aggregates can be >100µm in diameter. Small numbers of crystals are found within scattered hepatocytes. Occasionally surrounding the affected cells are clusters of free erythrocytes that obscure the parenchyma. Sinusoids in the adjacent areas are often congested. There is variable subcapsular hemorrhage present.

Lymph node: Cortical and medullary sinuses are expanded by moderate to large numbers of macrophages engorged with intracytoplasmic material identical to that described in the liver.

Contributor's Morphologic Diagnosis:

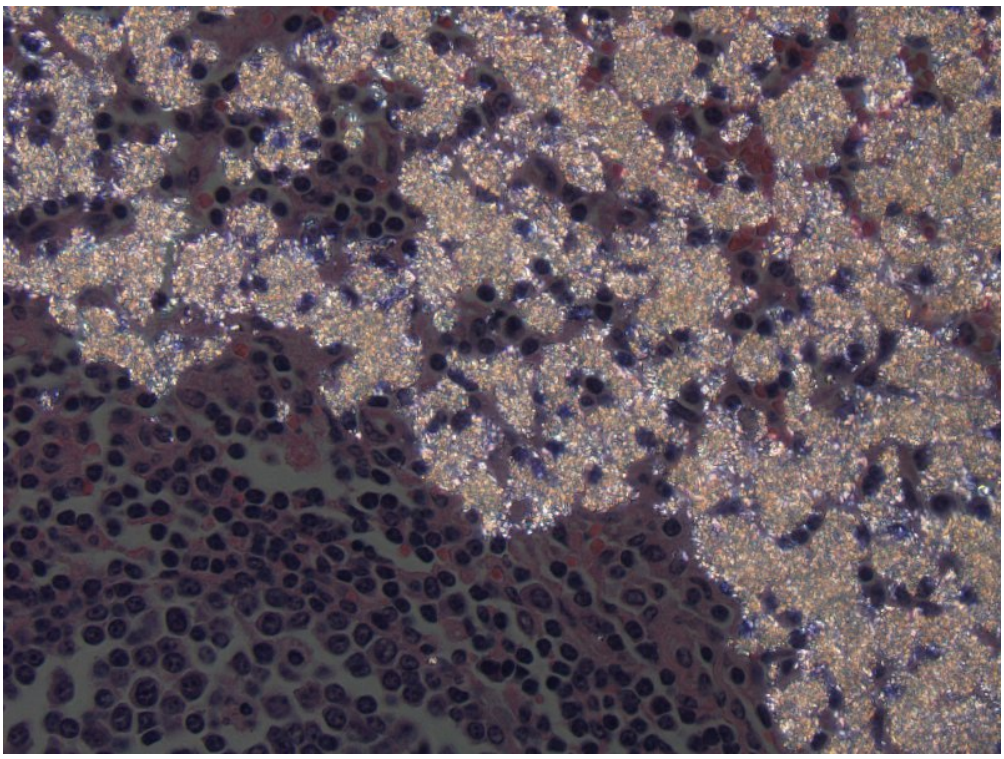
1. Liver: Accumulation of refractile material consistent with 2,8-dihydroxyadenine, *Bos taurus*, bovine.
2. Lymph node: Cortical and medullary histiocytic accumulation of refractile material consistent with 2,8-dihydroxyadenine.

Contributor's Comment: 2,8-dihydroxyadeninosis or "green liver disease" is a rare condition of bovines that is caused by the tissue deposition of 2,8-dihydroxyadenine (2,8-DHA), an insoluble green crystalline material. Accumulation of 2,8-DHA is most commonly seen in the liver and hepatic lymph nodes, but has also been reported in the kidney, and the renal, mediastinal, intercostal, and gastric lymph nodes.[7] The crystals appears as a light green to light tan mottling on the liver surface and parenchyma. Larger coalescing foci of 2,8-DHA are present in the hepatic lymph nodes. Renal lesions, if present, consist of light green mottling or streaking on the surface and on cut section or as concretions in the renal pelvis. The condition in bovines was initially reported about 20 years ago



Lymph node, ox. Higher magnification of pigment laden macrophages within the medullary sinuses.

after unusual green lesions were observed during postmortem examination at federally inspected abattoirs. Although the condition is rare, FSIS personnel are trained to recognize it since even rare conditions can be observed during the postmortem exam of the 30 million head of cattle that undergo federal inspection in the US every year.[9]



Lymph node, ox. The pigment is strongly birefringent. (HE, 400X)

Bovines with 2,8-dihydroxyadeninosis are thought to have an enzyme deficiency affecting purine catabolism. Normally, adenine is phosphorylated to adenine monophosphate in the first step of adenine catabolism. In the absence of the enzyme adenine phosphoribosyltransferase (APRT), adenine may instead be hydroxylated by the enzyme xanthine oxidase to 2,8-DHA, which precipitates in tissues as an insoluble crystal.[7] The light green to light tan color of the precipitate is retained throughout processing, embedding, and staining.

Deposits of 2,8-DHA in the renal tubules and lower urinary tract have been reported in humans, dogs, and APRT knockout mice. Uroliths and tubular casts can block the urinary tract, and lead to renal failure in these species.[5]

Other causes of grossly green discoloration in bovine tissues include green algal lymphadenitis (chlorellosis),[4] exogenous green pigment in lymph nodes draining tattoo ink from skin,[6] eosinophilic myositis,[10] green liver cell adenomas,[1,3] and bile imbibition. Green discoloration has been noted in injection site lesions in cuts of beef in modified-atmosphere packages.[8]

JPC Diagnosis:

1. Lymph node, medullary sinus: Sinus histiocytosis with intrahistiocytic birefringent pigment, breed unspecified (*Bos Taurus*), bovine.
2. Lymph node: Reactive lymphoid hyperplasia, diffuse, moderate.
3. Liver, portal areas: Histiocytosis, multifocal, mild with abundant intrahistiocytic birefringent pigment.

Conference Comment: An extremely rare condition, 2,8 dihydroxyadenine accumulation in cattle, has only been reported on once in veterinary literature,[7] but results in striking gross and microscopic

lesions that are worth mentioning. Adenine is a purine that is normally found in all tissues of the body (as it serves as one of the bases for the nucleic acids that form DNA and RNA) and is converted to adenylate by an enzyme called adenine phospho-ribosyltransferase. If this enzyme is lacking, adenine is excreted in the urine (resulting in crystalluria) or oxidized by xanthine oxidase to form 2,8-dihydroxyadenine. This substance is not soluble in water and at the body's pH precipitates to form crystals. Grossly, these crystals give tissues a light green hue and are most prominent around the portal triads of the liver and medullary sinuses of lymph nodes. When lymph nodes are incised, the surface of the knife becomes covered in a green, mucoid material. Rarely, there are light-green urinary calculi found within the renal pelvis or anywhere throughout the entirety of the lower urinary tract.[2] In this study[7], the most remarkable microscopic changes were in the portal triads of the liver, which were so enlarged by crystals, macrophages, and multinucleated giant cells that they comprised up to 30 percent of the hepatic mass. Crystal laden macrophages were also present in the medullary sinuses of regional lymph nodes. To date, the pathogenesis of 2,8-dihydroxyadenine accumulation has not been ascertained.

Contributing Institution:
National Centers for Animal Health, Ames, IA
www.ars.usda.gov/main/site_main.htm?modecode=36-25-30-00
www.aphis.usda.gov/nvsl

References:

1. Chu HH, Moon WS. Beta-catenin activated hepatocellular adenoma. *Clin Mol Hepatol*. 2013;19: 185-189.
2. Cianciolo RE, Mohr FC. Urinary system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol. 2. 6th ed. St. Louis, MO: Elsevier; 2016:429.
3. De Kock G, Fourie P. Green liver cell adenoma in a bovine. Digitized by the University of Pretoria, Library Services.
4. Hafner S, Brown CC, Zhang J. Green algal peritonitis in 2 cows. *Vet Pathol*. 2013;50:256-259.
5. Houston DM, Moore AE, Mendonca SZ, Taylor JA. 2,8-Dihydroxyadenine uroliths in a dog. *J Am Vet Med Assoc*. 2012;241:1348-1352.
6. Ladds PW. *A Colour Atlas of Lymph Node Pathology in Cattle*. Ames, IA: Iowa State University Press; 1986.
7. McCaskey PC, Rigsby WE, Hinton DM, Friedlander L, Hurst VJ. Accumulation of 2,8 dihydroxyadenine in bovine liver, kidneys, and lymph nodes. *Veterinary Pathology Online*. 1991;28:99-109.
8. Roeber DL, Belk KE, Engle TE, Field TG, et al. The effect of vitamin E supplementation on discoloration of injection-site lesions in retail cuts and the greening reaction observed in injection-site lesions in muscles of the chuck. *J Anim Sci*. 2003;81:1885-1894.
9. USDA-NASS: Livestock Slaughter 2012 Summary; 2013.
10. Vangeel L, Houf K, Geldhof P, De Preter K. Different *Sarcocystis* spp. are present in bovine eosinophilic myositis. *Vet Parasitol*. 2013;197:543-548.