Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2021-2022

Conference 1

18 August, 2021

Joint Pathology Center
Silver Spring, Maryland

CASE 1: 1 (4152313-00)

Signalment:
6-month-old male (intact) mastiff (*Canis familiaris*).

History:
The patient was referred to a veterinary medical teaching hospital following an acute, 1-day-history of lethargy and inappetence that quickly progressed to obtundation. Bloodwork performed at the rDVM revealed too high to read ALT, elevated ALP (805 U/L), hypoalbuminemia (2.7 g/dL) and hypoglycemia (23 mg/dL). PT and aPTT were both prolonged at >100 sec and >300 sec, respectively. Protein and bilirubin were detected on a urine dipstick. A SNAP test for Leptosperosis was negative. Despite aggressive palliative therapy, the patient continued to decline and euthanasia was elected.

Gross pathology:
Only gross lesions pertinent to the histologic findings and case discussion are provided. The entire length of the gastrointestinal tract contained a small amount of intralumenal, watery, dark red to black ingesta and fecal material (melena). The intestinal mucosa was mottled pale pink/red/dark purple. The hepatic parenchyma was diffusely friable and mottled pale/bright red with a prominent reticular pattern (Image). The renal cortex was diffusely dull tan/purple. The medullary parenchyma was disrupted by dozens of bright red to dull purple lines that radiated from the level of the renal crest through the medulla.

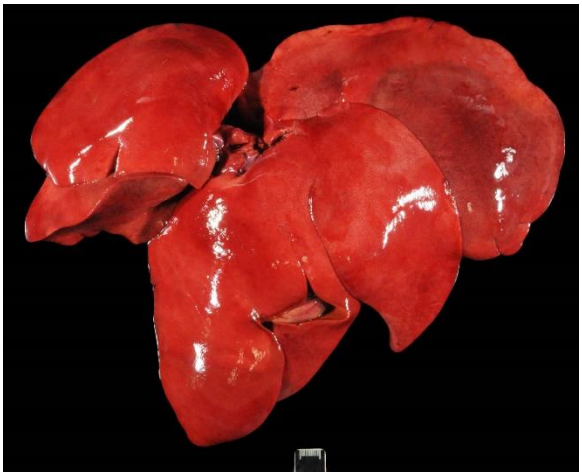

Figure 1-1. ***Liver, dog. The liver is diffusely friable and mottled pale/bright red with a prominent reticular pattern. (Photo courtesy of University of California, Davis Veterinary Medical Teaching Hospital, https://www.vetmed.ucdavis.edu/hospital).***

Laboratory results:
Amanita (Liver): Trace detected
Aflatoxin (Liver): None detected
Microcystins (Stomach contents): None detected

Microscopic description:

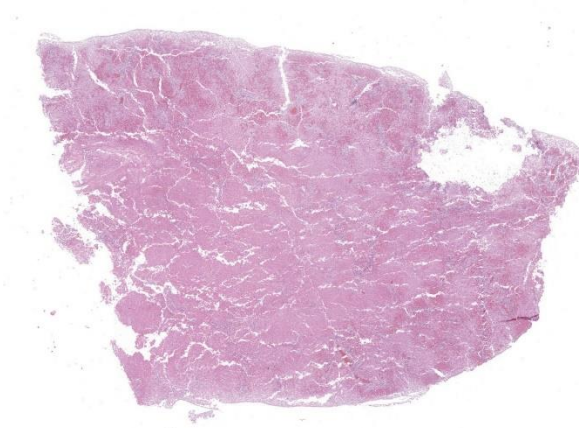

Figure 1-2. ***Liver, dog. A single section of liver is submitted for examination. At subgross magnification, there is loss of normal plate architecture, diffuse loss of vital staining, and many lobules contain hemorrhage. There are numerous cracks and fissures present within the sample as a result of hepatocellular necrosis and loss of tissue cohesion. (HE, 5X)***

The slide contains one section of hepatic parenchyma in which massive necrosis is characterized by dissociation of hepatic cords to complete loss of hepatocytes across all zones of multiple lobules. Lost hepatocytes are replaced by streams of extravasated red blood cells (hemorrhage), fibrin and karyorrhectic debris. Remaining individualized hepatocytes exhibit one or more of the following characteristics: cytoplasmic swelling, cytoplasmic vacuolation, cytoplasmic pigmentation, and nuclear pyknosis, karyorrhexis or karyolysis (degeneration and necrosis). Within the portal triads, portal collagen fibers are disrupted by increased clear space (edema), dissecting cords of hepatic progenitor cells (ductular reaction) and small numbers of lymphocytes and plasma cells. Scattered portal triads are additionally expanded by proliferating collagenous stroma (fibrosis) that breaches the limiting plate and spans portal regions (bridging portal fibrosis).

Contributor's morphologic diagnosis:
Liver: Massive hepatocellular necrosis with ductular reaction, hydropic degeneration, portal fibrosis and portal to portal bridging fibrosis.

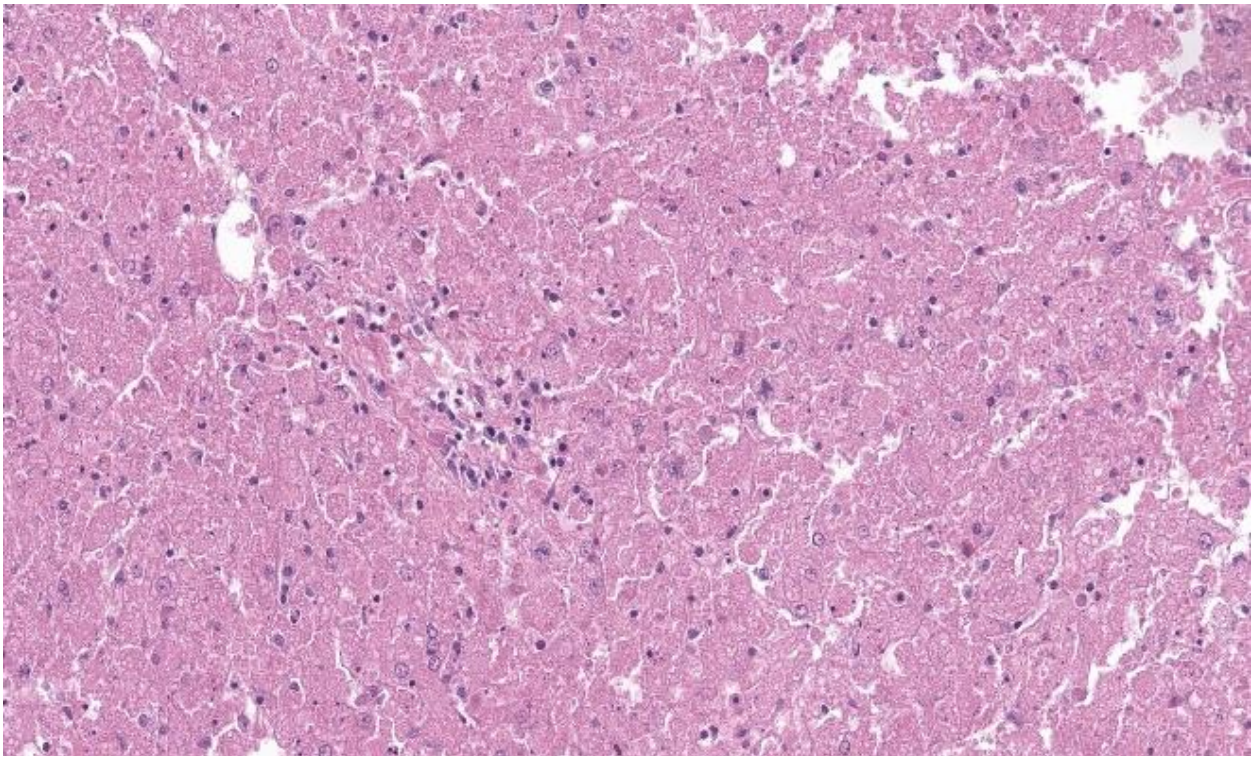

Figure 1-3. ***Liver, dog: Throughout the section, there is diffuse massive necrosis with hepatocellular individualization, rounding up, cytoplasmic vacuolation, and nuclear pyknosis and karyolysis. (HE, 400X)***

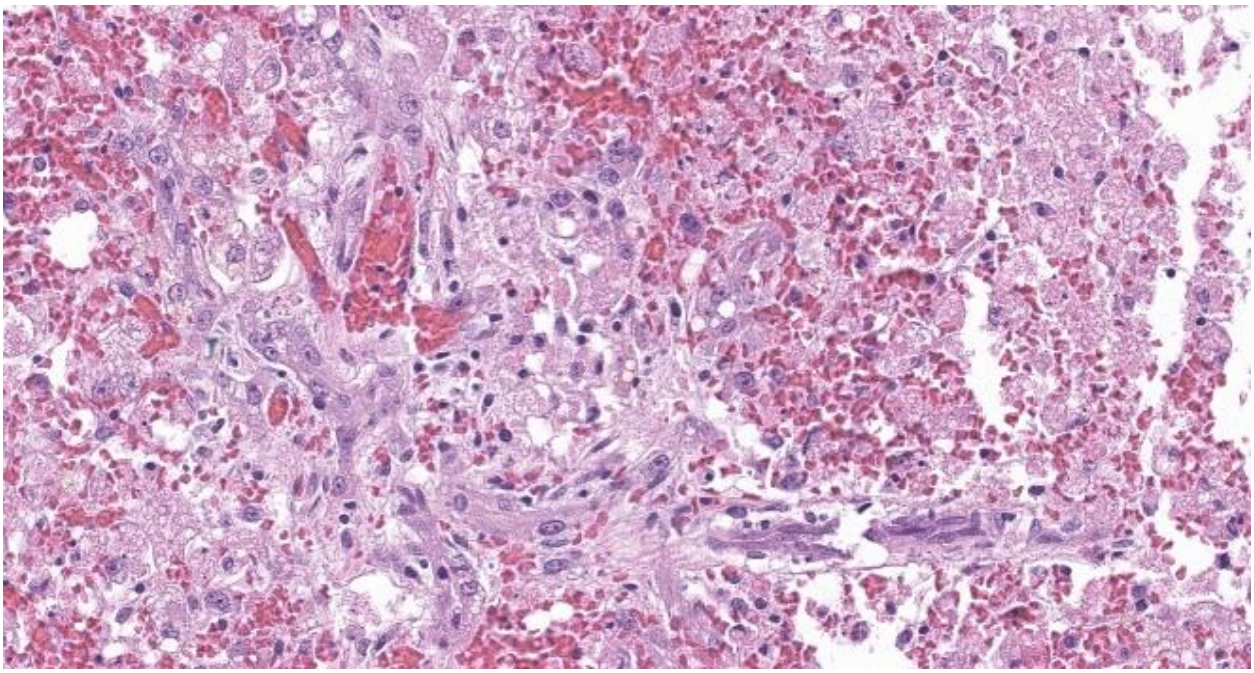

Figure 1-4. ***Liver, dog. There is moderate ductular reaction in response to widespread hepatocellular necrosis. (HE, 310X)***

Contributor's comment:
The constellation of the patient's age, clinical history, bloodwork abnormalities and hepatic lesions were most concerning for a toxic etiology. Fresh samples of hepatic parenchyma were submitted for toxicological analysis. A trace of amanitin was detected, therefore confirming ingestion of *Amanita phalloides*, which is also known as the death cap or death angel mushroom.

Amanitin poisoning is classically divided into four consecutive clinical stages, although each stage may not be evident in each case. Following ingestion, the first stage is characterized by approximately 8 to 12 hours of no clinical abnormalities. The patient will then develop severe gastrointestinal signs including vomiting and bloody diarrhea. The third stage of toxicity is characterized by brief clinical improvement, referred to as a "false recovery", and quickly followed by the 4th stage, which is characterized by multi-organ failure. The liver and kidneys appear to be most severely affected within this fourth stage, as evidenced by striking derangements in bloodwork and urinalysis.

Patients typically die within 12 to 84 hours following ingestion of the lethal dose. The lethal dose (LD) for dogs is estimated to be around 0.5 mg/kg, which may be contained within 20 g of Amanita mushrooms.[5] Therefore, one mushroom cap can provide a lethal dose to dogs. Evaluation of numerous treatment options has remained fruitless as the mortality rates in dogs remains high. Interestingly, studies have revealed remarkable variance in species sensitivity.[4] For instance, the rate of gastrointestinal absorption is much greater in dogs than in mice and rabbits. Furthermore, rats appear to be relatively resistant to amanitin's toxic effects.

Amanitin's mechanism of action primarily stems from the toxin's ability to inhibit RNA polymerase II. Ceased transcription and decreased protein synthesis eventually leads to cell death. Cells with high metabolic rate, such as hepatocytes, crypt cells and proximal convoluted tubules, are the most severely affected, thus explaining the classic triad of hepatic failure, gastroenteritis and renal failure, respectively. In addition to inhibition of RNA polymerase II, amanitin may induce hepatocellular apoptosis, thus compounding hepatocellular death and hepatic failure.[2]

Given the patient's clinical history and the hepatic lesions, top differential diagnoses included blue-green algae poisoning

(microcystins) and aflatoxin. However, no microcystins were detected within the patient's stomach content nor was any aflatoxin detected within the fresh samples of hepatic parenchyma.

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JPC diagnosis:
Liver: Hepatocellular necrosis, massive, diffuse, with ductular reaction and stromal collapse.

JPC comment:
The contributor provides an excellent overview of the pathogenesis and clinical progression of amanitin toxicosis following ingestion of *Amanita phalloides*.

A. phalloides is found worldwide due to the importation of trees from its native Europe. An ectomycorrhizal fungus, *A. phalloides* shares a symbiotic relationship with the roots of trees found in both deciduous and coniferous forests. In the United States the mushroom is most commonly found alongside oak trees on the west coast in contrast to the east coast where it is more commonly found alongside pine trees.[1] The large fruiting bodies appear in the late summer and fall and have a smooth, yellowish-green to yellowish-brown cap, white gills, a white ring around the upper part of the stem (veil), and a white cup-like structure around the base of the stem (volva).[4]

Amatoxins are heat-stabile bicyclic octapeptides that include the amanitins (α-, β-, γ-, and ε-amanitins), amanin, amanullin, and proamanullin and have been isolated from several genera of mushrooms, including *Amanita, Galerina* and *Lepiota*.[2,3] α-Amanitin is the most toxic of eight known amanitins and is found in 38 species of mushroom from the genera *Amanita*, including *A. phalloides*.[1,2]

Early diagnosis of amanitin toxicosis is frequently hindered as ingestion of the mushroom is often unwitnessed and veterinary care is not pursued until development of gastrointestinal signs or multi-organ failure.[1] A recent study evaluating 59 cases of α-amanitin toxicosis in dogs found upon presentation, alanine transferase activity (ALT) was mildly to markedly increased in 97% of dogs, hypoglycemia in 78% and coagulation times were increased in 91%.[1] Although the signs of liver failure are not specific to α-amanitin toxicosis, early onset hypoglycemia was found to be a distinguishing biochemical abnormality and its identification helped distinguish affected dogs from those with other causes of gastroenteritis; however, differential diagnoses should also include xylitol toxicosis, severe anaphylaxis, sepsis, and portosystemic shunt.[1]

During necropsy, the liver is often swollen, without any other significant abnormalities and is histopathologically characterized by massive hepatocellular necrosis with collapse of hepatic cords and acute tubular necrosis in dogs that develop renal failure. [4]

Confirmatory diagnosis at select veterinary toxicology laboratories is made by detection of α-amanitin in submitted samples of serum, urine, gastric contents, suspected mushroom, liver or kidney. Serum and urine samples should be collected and frozen at various points beginning as early as possible following exposure.[4]

Finally, there was spirited discussion amongst participants in regard to the associated ductular reaction and its possibility of being secondary to an unrelated process due to the relatively rapid progression of hepatocellular necrosis observed in cases of amanitin toxicity.

References:

1. Kaae JA, Poppenga RH, Hill AE. Physical examination, serum biochemical, and coagulation abnormalities, treatments, and outcomes for dogs with toxicosis from α-amanitin-containing mushrooms: 59 cases (2006-2019). *J Am Vet Med Assoc*. 2021;258(5):502-509.

2. Magdalan J, Ostrowska A, Piotrowska A, et al. Alpha-amanitin induced apoptosis in primary cultured dog hepatocytes. *Folia Histochemica et Cytobiologica*. 2010;48:58-62.
3. Puschner B, Rose HH, Filigenzi MS. Diagnosis of Amanita toxicosis in a dog with acute hepatic necrosis. *J Vet Diagn Invest*. 2007;19(3):312-317.
4. Puschner B, Wegenast C. Mushroom poisoning cases in dogs and cats: Diagnosis and treatment of hepatotoxic, neurotoxic, gastroenterotoxic, nephrotoxic and muscarinic mushrooms. *Veterinary Clinics of North America: Small Animal Practice*. 2012;42:375-387.
5. Spoerke David G., Rumack Barry H. *Handbook of mushroom poisoning: diagnosis and treatment.* Bosa Roca, United States: Taylor and Francis Inc.; 1994.
6. Lewis HL, Kleiner DE. Hepatic injury due to drugs, herbal compounds, chemicals, and toxins. In: Burt AD, Ferrell LD, Portmann BC, eds. *MacSween's Pathology of the Liver*. 6th ed. Edinburgh: Churchhill Livingstone/Elsevier; 2012:690.

CASE 2: S15_8036 (4084865-00)

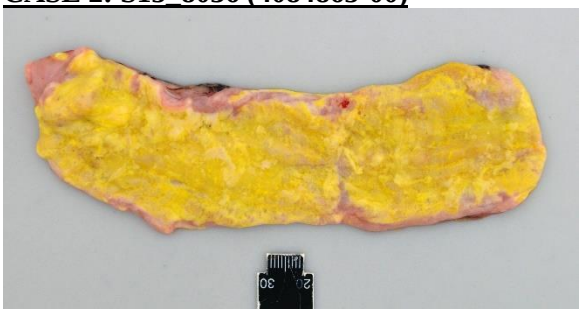

Figure 2-1. ***Small intestine, calf. Intestinal contents are greasy and yellowish. (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012, Bern, Switzerland http://www.itpa.vetsuisse.unibe.ch/)***

Signalment:
2-month-old, female, red Holstein calf, *Bos taurus, bovine.*

History:
The calf was referred to the ruminant clinic with severe emaciation, failure to thrive and long lasting, intermittent diarrhea resistant to therapy. Routine viral, bacteriological and parasitological examinations (Rotavirus, Coronavirus, Salmonella, E.coli, Cryptosporida, Coccidia) were negative. Blood chemistry revealed that cholesterol and triglyceride levels were severely lower (0.18 and 0.06 mmol/L) when compared to healthy animals (1.79-3.20 and 0.27-0.40 mmol/L) in the same age range[6]. The animal was euthanized because of poor prognosis and suspicion of a genetic defect as a possible cause for the clinical symptoms.

Gross Pathology:

Figure 2-2. ***Small intestine, calf. The mucosa appears whitish and is thickened by edema. (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012, Bern,***

The calf was cachectic and the perianal region was smeared with yellow to green colored feces.

The small and large intestine were diffusely filled with a moderate to large amount of foamy and greasy, liquid content consistent with steatorrhea. The color varied from bright yellow in the proximal parts of the gastro-intestinal tract, to more green color distally. The mucosa, especially of the jejunum, was diffusely edematous and appeared thickened and whitish.

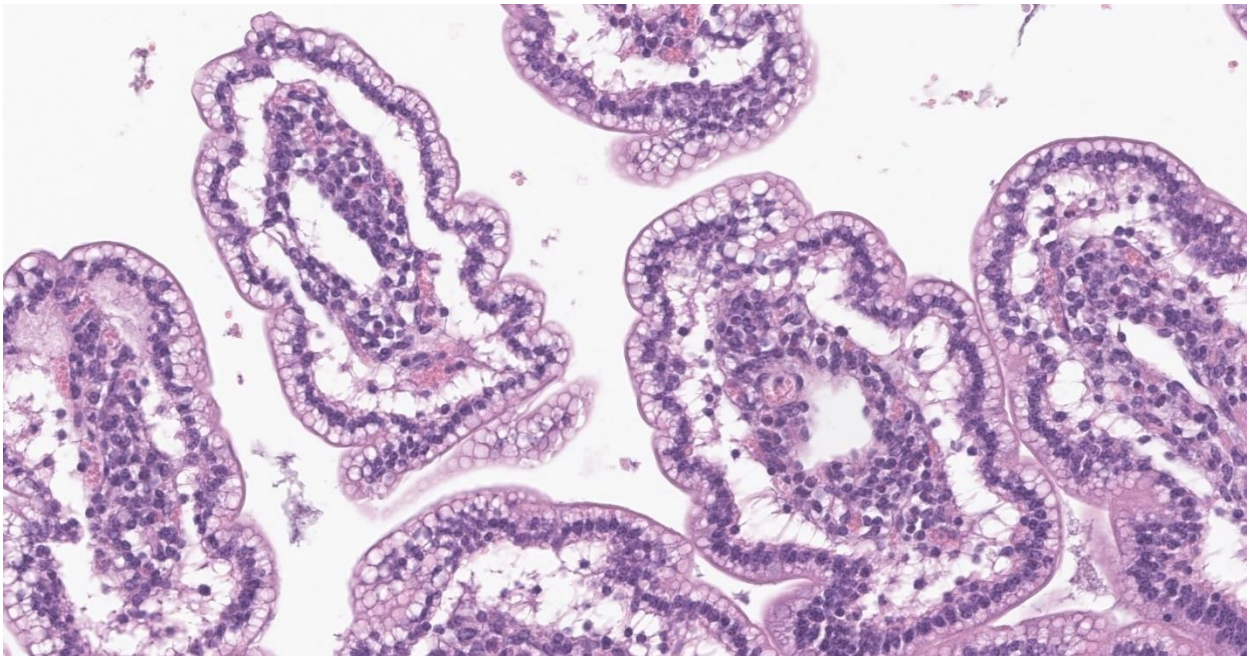

Figure 2-4. ***Jejunum, calf. Villar enterocytes contain numerous lipid vacuoles within their apical cytoplasm There is coalescing edema lifting the enterocytes off of the underlying lamina propria and multifocal lacteal dilation. (HE, 315X)***

Laboratory results:
Genetic analysis revealed a 1.3 kb insertion within the coding sequence of the apolipoprotein B (*APOB*) gene.

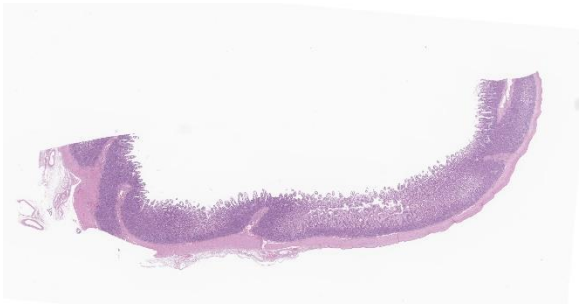

Figure 2-3. ***Jejunum, calf. Subgross lesions in the submitted section of jejunum are limited to multifocal markedly villar lacteal dilation. (HE, 8X)***

Microscopic description:
Small intestine (jejunum): Diffusely within the enterocytes covering the tips of the villi, optically empty, round to oval vacuoles varying from approximately 2 to 20 µm in diameter are present (fat vacuoles). Occasionally the vacuoles are located subnuclearly and dislocate the nucleus to the apical cell border. Within the lamina propria, multifocal to coalescing areas of loose, optically empty spaces (edema) are present and the lacteals are dilated. The amount of lymphocytes within the submucosa is slightly increased.
The optically empty vacuoles stain positive by the Sudan stain for lipids in frozen sections.

Transmission electron microscopy (Figure 2-6) corroborated the presence of large intracytoplasmic lipid droplets in the small intestinal enterocytes. The droplets filled most of the supranuclear cytoplasm but also occurred in an infranuclear position (Fig. EMCD1).

Contributor's morphologic diagnosis:
Small intestine (jejunum) HE and TEM: Diffuse, severe, intracytoplasmic vacuolization (lipid retention) with moderate, diffuse edema.

Contributor's comment:
The inherited autosomal recessive genetic defect affecting Holstein calves, named cholesterol deficiency (CD), was reported for the first time in the Summer of 2015 in Germany.[5] Homozygous calves demonstrate clinical signs of diarrhea unresponsive to treatment and failure to thrive.[5] They suffer marked hypocholesterolemia and hypolipidemia, indicating an inherited fat metabolism disorder. These animals usually die within the first six months of life and it

has been assumed that about 80% of homozygous affected calves do not survive more than one year.[5] Heterozygous carrier animals do not show any clinical signs but have reduced levels of blood cholesterol and triglycerides.[5] Breeding organizations in Switzerland and other countries have reported an increasing occurrence of cases in Holstein cattle. The causal mutation has recently been identified in the apolipoprotein B gene (*APOB*).[7] This case is one out of the series describing the pathological phenotype of CD for the first time.[8]

Pedigree analysis revealed the Canadian Holstein bull Maughlin Storm as the first carrier for this disorder.[5,7] The mutation in this case represents a 1.3 kb insertion of a transposable LTR element (ERV2-1) in the coding sequence of the *APOB* gene, which leads to truncated transcripts and aberrant splicing.[7]

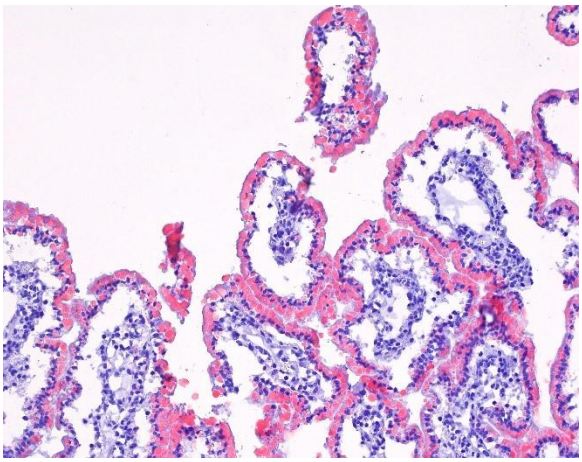

Figure 2-5. ***Jejunum, calf. An Oil Red O stain on a frozen section of jejunum demonstrates the lipid in the villar enterocyte cytoplasm. (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012, Bern, Switzerland http://www.itpa.vetsuisse.unibe.ch/) (Oil Red O, 200X)***

The encoded apolipoprotein B (APOB) protein is an essential apolipoprotein of chylomicrons and low-density lipoproteins. The mutation represents a loss of function mutation, similar to the autosomal recessive inherited familial hypobetalipoproteinemia-1 (FHBL1) in humans.[7] The *APOB* gene encodes two proteins via a mRNA editing process: the APOB-48 protein is required for chylomicron production and cellular transport of lipids in the small intestine, and the APOB-100 protein which is expressed in the liver. The APOB-100 protein is a structural component of very low density lipoprotein (VLDL) and its metabolic products, and serves as the ligand for low density lipoprotein (LDL)-receptor mediated endocytosis of lipid particles.[3]

Truncation mutations in the APOB-48 protein, which is exclusively synthesized in the enterocytes of the small intestine[4], were shown to result in defective chylomicron formation in the small intestine.[3,9] The histologically visible accumulation of lipid vacuoles within the enterocytes in the CD-affected cattle indicates that the enterocytes of these animals are capable of resorbing fat from the ingesta. However, the hypocholesterolemia and low triglyceride concentrations demonstrate that the transfer of these lipids from the enterocyte into the blood is impaired or even absent. The clinical and pathological findings support this mechanism. In human patients, FHBL1 is additionally characterized by chronic malabsorption of lipid-soluble vitamins, leading to retinal degeneration, neuropathy and coagulopathy.[6]

The gross findings include steatorrhea (diarrhea) and an edematous mucosa of the small intestine. The animals are usually cachectic. The histological findings are limited to the small intestine and consist of lipid vacuoles within the enterocytes as well as dilated lacteals.[8]

Taking the pathological findings of steatorrhea, the histological and TEM phenotype of lipid vacuole accumulation within the enterocytes of the tips of the villi in the small intestine and the clinical symptoms of hypocholesterolemia, low triglyceride concentration and the neurological symptoms into account, bovine CD is highly similar to human FHBL.[3,9]

The main limitations for the diagnosis of CD in cattle are the unspecific clinical symptoms of diarrhea and failure to thrive in young calves. It is important to first exclude common agents causing diarrhea. In case of intermittent diarrhea resistant to treatment in young Holstein cattle, analysis of total cholesterol and triglycerides can lead to a suspicion of CD. Pathological investigations are only diagnostic if samples of

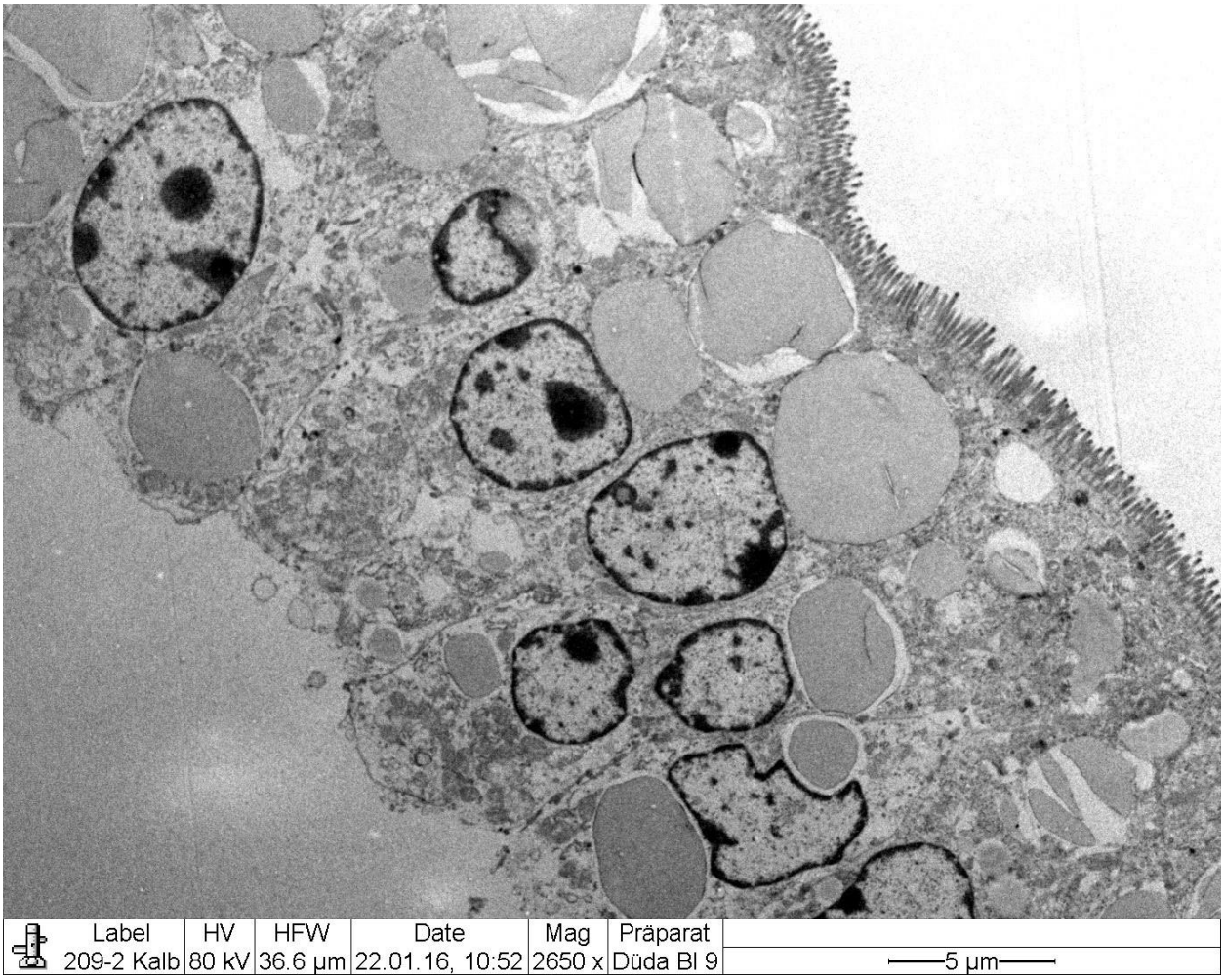

Figure 2-6. ***Jejunum, calf. An ultrastructure photograph demonstrates numerous lipid droplets free in the cytoplasm of enterocytes. (Photo courtesy of: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Laenggassstrasse 122, CH-3012, Bern, Switzerland http://www.itpa.vetsuisse.unibe.ch/)***

the small intestine are immediately fixed in formalin (within minutes after euthanasia). Otherwise, the lipid vacuoles in the enterocytes of the villi tips are lost due to immediate autolytic changes in the small intestine. A clinical or pathological suspicion of CD should be confirmed using a gene test detecting the *APOB* gene mutation after consideration of pedigree information indicating inbreeding linked to the founder sire Maughlin Storm.[8]

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JPC diagnosis:
1. Small intestine: Villar enterocyte lipidosis, diffuse, marked, with lacteal dilation.

2. Adipose tissue: Atrophy, diffuse, moderate.

JPC comment:
The contributor provides an excellent overview of the homozygous apolipoprotein B gene (*APOB*) mutation associated with cholesterol deficiency in Holstein calves.

When initially described in 2016, the APOB mutation was only thought to induce clinical signs in homozygous animals, although heterozygotes were noted to have reduced levels of blood cholesterol and triglycerides.[5] A subsequent study identified 18 cases of

heterozygous Holstein calves with history of poor development, low weight, and intermittent diarrhea with concurrent reduced cholesterol and triglyceride blood concentrations.[2] These animals tested negative for rotavirus, coronavirus, bovine viral diarrhea virus, coccida, cryptosoporida, *E. coli*, and *Salmonella* spp. and symptomatic treatments did not lead to clinical improvement. Given the reduced cholesterol and triglyceride blood concentrations and lack of other plausible explanation, these cases likely indicate the mutation acts as incomplete dominance with reduced penetrance in heterozygotes.[2]

An additional study evaluating the lactational and reproductive performance of heterozygous Holstein cows predictably identified markedly lower circulating levels of cholesterol and cholesterol in lipoprotein fractions compared to noncarriers.[1] However, lactational and reproductive performance was not impaired compared to noncarriers. The authors therefore found no need to eradicate APOB carriers from production, though risk-matings of carriers should be avoided.[1]

References:

1. Gross JJ, Schwinn AC, Schmitz-Hsu F, et al. The APOB loss-of-function mutation of Holstein dairy cattle does not cause a deficiency of cholesterol but decreases the capacity for cholesterol transport in circulation. *J Dairy Sci*. 2019;102(11):10564-10572.
2. Häfliger IM, Hofstetter S, Mock T, et al. APOB-associated cholesterol deficiency in Holstein cattle is not a simple recessive disease. *Anim Genet*. 2019;50(4):372-375.
3. Hooper AJ, van Bockxmeer FM, Burnett JR. Monogenic hypocholesterolaemic lipid disorders and apolipoprotein B metabolism. Crit Rev Clin Lab Sci 2005;42:515–545.
4. Kane JP, Hardman DA, Paulus HE. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. Proc Natl Acad Sci U S A 1980;77:2465–2469.
5. Kipp S, Segelke D, Schierenbeck S, et al. A new Holstein Haplotype affecting calf survival. *Interbull Bull* 2015; 49:49-53
6. Lee J, Hegele RA. Abetalipoproteinemia and homozygous hypobetalipoproteinemia: a framework for diagnosis and management. *J Inherit Metab Dis* 2014;37:333–339.
7. Menzi F, Besuchet-Schmutz N, Fragnier M, et al. A transposable element insertion in APOB causes cholesterol deficiency in Holstein cattle. *Anim Genet* 2016; 47:253-257.
8. Mock T., Mehinagic K., Menzi F., et al. Clinicopathological Phenotype of Autosomal Recessive Cholesterol Deficiency in Holstein Cattle. *J Vet Intern* Med 2016 Submitted January 27, 2016; Revised March 14, 2016; Accepted.
9. Welty FK. Hypobetalipoproteinemia and abetalipoproteinemia. *Curr Opin Lipidol* 2014;25:161–168.

CASE 3: S1601220 (4101207-00)

Signalment:
8-month-old, female Romney lamb, *(Ovis aries).*

History:
This lamb was found dead and was subsequently presented to Massey University Veterinary Pathology Department for necropsy.

Gross Pathology:
The lamb was in poor body condition and was underweight for its age. It had marked icterus of the subcutaneous connective tissues and mucous membranes. The pancreas was small, nodular and firm. Widespread crusting and ulceration were present on the ear pinnae and the skin surrounding the eyes and muzzle consistent with acute facial eczema. Four slow-release 30 g zinc boluses were present in the ruminal contents.

Laboratory results:
Liver: Zinc 161 mg/kg (Reference range 25-100 mg/kg)

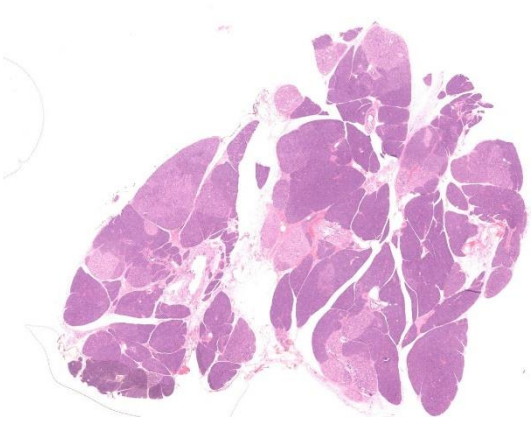

Figure 3-1. ***Pancreas, sheep. A section of pancreas is submitted for examination. At subgross magnification, numerous lobules demonstrate a loss of normal architecture and stain affinity. (HE, 5X)***

Microscopic description:

Pancreas: Within the pancreas, individual lobules show well-demarcated and varying degrees of necrosis and fibrosis. Mildly affected lobules are characterized by infiltration of the parenchyma with small numbers of neutrophils and mononuclear cells. Individual acinar cells appear shrunken with loss of zymogen granules and pyknotic nuclei. In moderately affected lobules, acinar cells show variable loss of basophilia and zymogen granules. Individual cells are swollen and vacuolated (degenerate), with other cells appearing shrunken and hypereosinophilic with pyknotic nuclei (necrosis). Acini and interlobular ducts are multifocally dilated, with enlarged luminal spaces, some containing small numbers of neutrophils. The parenchyma is infiltrated by neutrophils and mononuclear cells with corresponding disorganization and loss of associated pancreatic acini and ducts.

In severely affected lobules, the intralobular parenchyma is extensively replaced by fibrous connective tissue and fibroblasts. The few remaining acini show marked loss of basophilia and zymogen granules, and frequently contain dilated cystic luminal spaces with attenuated acinar cells. Moderate numbers of mononuclear cells and fewer neutrophils are present throughout the parenchyma.

The interlobular spaces are moderately expanded by multifocal areas of haemorrhage and oedema, and there is marked interlobular fibrosis.

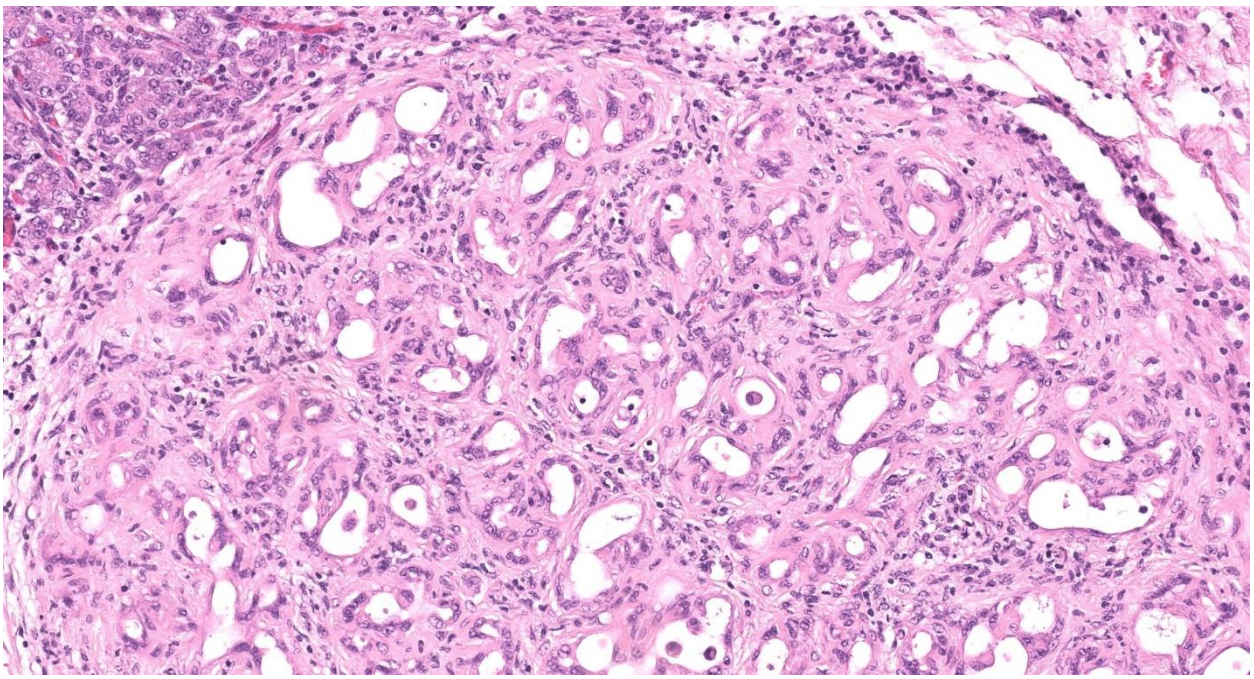

Figure 3-2. ***Pancreas, sheep. Severely affected lobules are devoid of acinar tissue, with only ducts remaining. The interstitium is expanded by loosely arranged collagen, numerous fibroblasts, and scattered aggregates of lymphocytes and plasma cells. (HE, 211X)***

Contributor's morphologic diagnosis:
Pancreas: Degeneration and necrosis, chronic-active, multifocal, severe, with intralobular and interlobular fibrosis, ovine.

Contributor's comment:
In this sheep, the diagnosis of zinc toxicity was made on the basis of similar clinical and pathological findings compared to reported experimental and natural cases of zinc toxicity, the reported increased zinc liver concentrations and the presence of four zinc bullets within the rumen.

Zinc is a trace element which has been shown to produce toxic effects in multiple species including humans, dogs, sheep, cattle and many wildlife species.[1,6] In ruminants, zinc toxicity may result from administration of zinc as protection against sporidesmin toxicity.[1,9,13] In other animals, zinc toxicity typically results from dietary indiscretion, and may be caused by the ingestion of zinc-coated U.S. pennies, batteries, paint, hardware, creams, and automotive parts.[2,6]

The largest effects are predominantly in the exocrine pancreas, where zinc excretion occurs, but other organs, including the kidney, liver, abomasum, small intestine and blood, may be affected.[1,11] Clinical signs include anorexia, lethargy, vomiting and may include intravascular haemolysis, haemoglobinuria, and icterus.[1,6] In large animals, poor weight gains may be the most prominent clinical sign.

In ruminants, initial effects are produced within ductular structures within the exocrine pancreas with subsequent changes in acinar cells. The endocrine pancreas is not affected. The initial reported lesions include vacuolar degeneration, necrosis and regeneration with a pronounced lobular distribution. With prolonged zinc intoxication, pancreatic necrosis exceeds the rate of regeneration, with fibrosis and atrophy becoming the predominant findings.[1,14]

As seen in this case, relatively normal appearing lobules may be present alongside severely affected lobules. It is hypothesized when injury of a duct occurs, drainage is impeded causing damage to the that lobule.[14] This is supported by the fact that cattle, which have a dual exocrine pancreatic drainage via an accessory duct, do not show a discrete lobular pattern despite having other similar histopathological changes.[14] Tissue zinc concentrations in experimental studies in sheep with similar pancreatic lesions have been reported at over 800 mg/kg, [14] and a value of 161 mg/kg, which was found in this lamb, is outside the reference range, but on low end of previously reported concentrations in experimentally induced zinc toxicity in sheep. Additionally, zinc induces a Heinz body haemolytic anaemia of which the exact mechanism is unknown. Possible mechanisms include hapten-induced immune-mediated destruction, direct cell membrane damage, or erythrocyte enzyme inhibition. Released haemoglobin may result in acute renal tubular injury, in addition to hypoxic injury caused by anaemia and reduced renal blood flow.[4]

Zinc salts have been shown to provide protection against sporidesmin toxicity in sheep, by forming a stable mercaptide with sporidesmin, thereby preventing the formation of free radicals from this toxin.[10,12,16] Sporidesmin, a mycotoxin produced by Pithomyces chartarum, induces damage to biliary epithelium, resulting in cholestasis, and in chronic cases, hepatic fibrosis, atrophy and nodular regeneration. Phylloerythrin, a metabolite of chlorophyll, is unable to be excreted via damaged bile ducts and accumulates in the skin resulting in photodermatitis. Prior damage to the liver caused by sporidesmin toxicity has been reported to be associated with an increased likelihood of zinc toxicity.[15] Metallothionein which is involved in detoxifying metal ions, is produced by the liver. In addition, serum albumin, which binds zinc in the blood, is also produced by the liver, and both albumin and metallothionein levels may be reduced in sheep with liver damage.[15]

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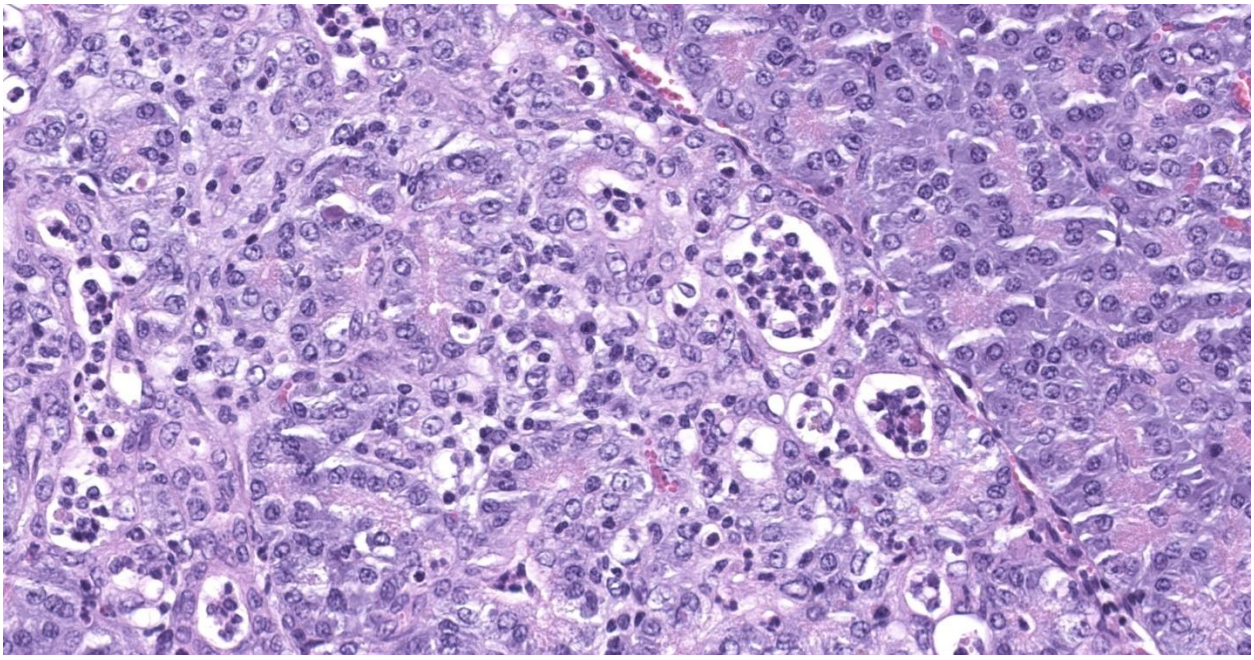

Figure 3-3. ***Pancreas, sheep. Less affected lobules demonstrate loss of normal acinar architecture with acinar cell degeneration, necrosis, and minimal regeneration (as compared to adjacent largely unaffected lobule at left). Degenerating acinar cells are shrunken with loss of zymogen granules. Neutrophils are present both within the interstitium and affected acini. (HE, 278X)***

JPC diagnosis:
Exocrine pancreas: Degeneration and necrosis, lobular, multifocal, marked, with tubular complexes and fibrosis.

JPC comment:
The contributor provides an excellent review of zinc toxicosis and its pathologic effects on the pancreas.

A previous study comparing natural and experimental cases of zinc toxicity in sheep found pathological changes may be observed in any organ involved in the absorption, excretion, or intermediary metabolism of zinc, including the abomasum, small intestine, liver, and kidney addition to the rumen and adrenal glands; however, the pancreas was the only organ consistently affected.[1] This is significant in that this organ is often overlooked by pathologists, particularly when examining ruminents.[1]

A separate zinc related illness known as Metal fume fever (MFF) has been described in the human literature and was first described in metal workers during the mid-19th century, predominantly affecting foundry workers and welders of galvanized steel.[18] The syndrome occurs due to the inhalation of freshly formed oxide fumes (predominately in the form of zinc oxide) generated from molten bronze and the welding of galvanized steel, and continues to affect an estimated minimum of 1,500-2,000 of the approximately 700,000 metal workers in the United States each year.[18] Also known as "zinc shakes" and first described as "brass founders' ague", MFF results in a "flulike" illness due to the inhalation of freshly formed ZnO, resulting in numerous pro-inflammatory changes including the production of pro-inflammatory cytokines and recruitment of inflammatory cells into the lungs and is characterized by fever, cough, wheezing, chest tightness, chills, myalgia, leukocytosis with a left sift, thirst, metallic taste, and salivations.[18] The diagnosis is typically based on clinical findings and history of occupational exposure and resolution is spontaneous. Treatment is symptomatic, with an emphasis on prevention of subsequent exposure. Interestingly, smoking has been found to modify the effect of welding fumes on specific markers of inflammation, with non-smokers experiencing a significant increase in circulating WBC counts in comparison to smokers.[18]

References:

1. Allen J, Masters H, Peet R, et al. Zinc toxicity in ruminants. *Journal of Comparative Pathology*. 1983; 93: 363-377.
2. Boes KM, Durham AC: Bone Marrow, Blood Cells and the Lymphoid/Lymphatic System. In: Zachary JF, ed. *Pathologic Basis of Veterinary Disease*. 6th ed. St. Louis, MO: Elsevier; 2017: 746-747.
3. Campbell J, Mills C. The toxicity of zinc to pregnant sheep. *Environmental research*. 1979;20: 1-13.
4. Cianciolo RE, Mohr FC: Urinary system. In: Maxie MG, ed. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals*. 6th ed. St Louis, MO: Elsevier; 2016: 356-357.
5. Graham T, Holmberg C, Keen CL, Thurmond M, Clegg M. A pathologic and toxicologic evaluation of veal calves fed large amounts of zinc. *Veterinary Pathology*. 1988;25: 484-491.
6. Gurnee CM, Drobatz KJ. Zinc intoxication in dogs: 19 cases (1991-2003). *Journal of the American Veterinary Medical Association*. 2007;230: 117 4-1179.
7. Jubb KVF, Stent AW. Pancreas. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 2, 6th ed. St. Louis, MO: Elsevier; 2016:356- 357.
8. LaBonde J: Toxicity in pet avian patients. In: *Seminars in Avian and Exotic Pet Medicine*, pp. 23-31. Elsevier, 1995.
9. Munday R, Thompson AM, Fowke EA. et al. A zinc-containing intraruminal device for facial eczema control in lambs. *New Zealand Veterinary Journal*. 1997;45: 93- 98.
10. Munday R. Studies on the mechanism of toxicity of the mycotoxin sporidesmin. I. Generation of superoxide radical by sporidesmin. *Chem. Biol. Interact*. 41, 361-374, 1982
11. Munday R. Studies on the mechanism of toxicity of the mycotoxin sporidesmin. 2-evidence for intracellular generation of superoxide radical from sporidesmin. *J. Appl. Toxicol*. 4, 176-181, 1984
12. Munday R. Studies on the mechanism of toxicity of the mycotoxin sporidesmin 3-inhibition by metals of the generation of superoxide radical by sporidesmin. *J. Appl. Toxicol*. 4, 182-186, 1984
13. Smith B. Toxicity of zinc in ruminants in relation to facial eczema. *New Zealand Veterinary Journal*. 1977;25: 310-312.
14. Smith B, Embling P. Sequential changes in the development of the pancreatic lesion of zinc toxicosis in sheep. *Veterinary Pathology*. 1993;30: 242-247.
15. Smith BL, Embling PP. Effect of prior sporidesmin intoxication on the pancreopathy associated with zinc oxide toxicity. *New Zealand Veterinary Journal*. 1999;47: 25-27
16. Smith BL, Embling PP, Towers NR, Wright DE, Payne E. The protective effect of zinc sulphate in experimental sporidesmin poisoning of sheep. *New Zealand Veterinary Journal*. 1977;25: 124-127.
17. Valli VEO, Kiupel M, Bienzle D: Hematopoietic system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol 3. 6th ed. St. Louis, MO: Elsevier; 2016:125-126.
18. Wardhana, Datau EA. Metal fume fever among galvanized welders. *Acta Med Indones*. 2014;46(3):256-262.

CASE 4: K18-1448 (4127786-00)

Signalment:
15.5 year old castrated domestic shorthair cat (*Felis catus*)

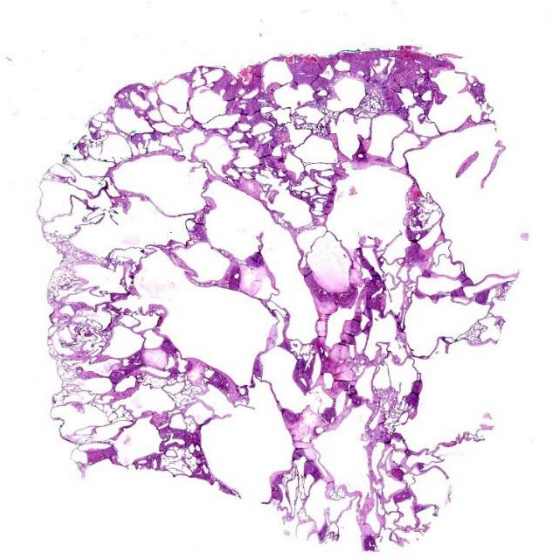

Figure 4-1. ***Liver, cat. The submitted section of liver is expanded by numerous ectatic bile ducts (HE, 5X)***

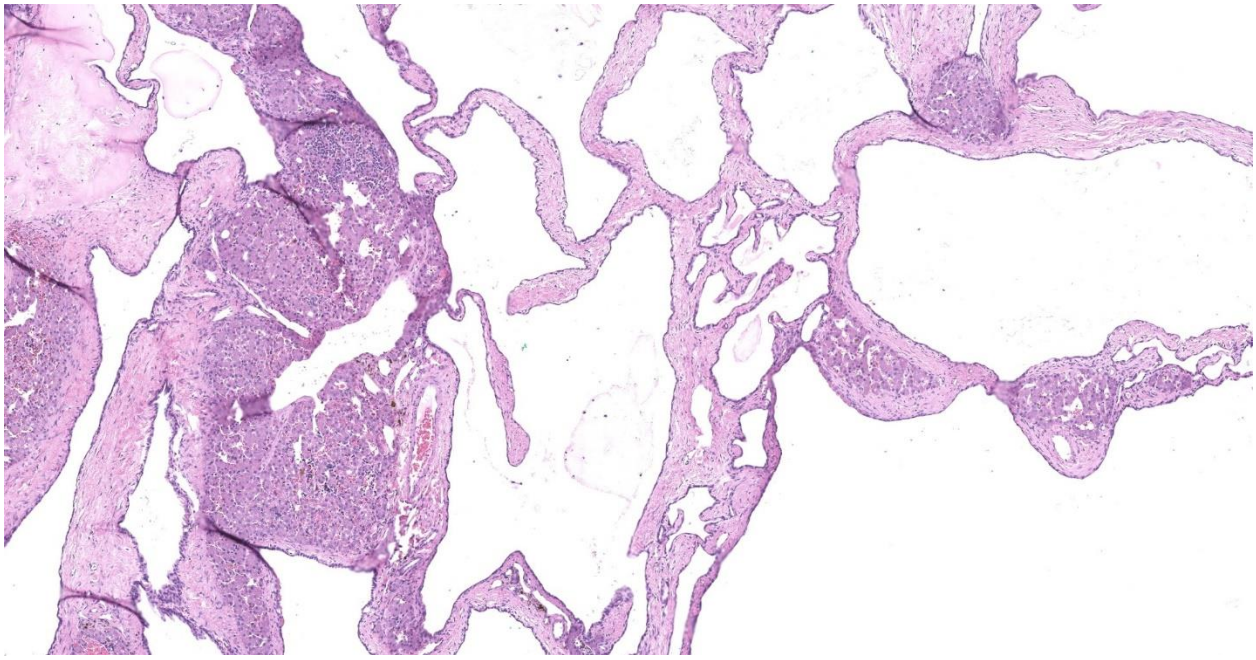

Figure 4-2. ***Liver, cat. Ectatic bile ducts are separated by dense fibrous stroma which often contains islands of entrapped hepatocytes. (HE, 62X)***

History:
An adult cat was treated medically after he was presented for loss of appetite and lethargy. Five days later the cat represented with no change in status. Following fluid therapy and another two days, the cat was examined by ultrasound. An intestinal foreign body was diagnosed and abdominal surgery performed. In addition to removing an unspecified foreign body from small intestine, the surgeon excised a discreet cystic mass on the liver. It was submitted for diagnosis. The cat made an uneventful recovery.

Gross Pathology:
The submitted 54 x 40 x 25 mm multilocular cystic mass released clear water-like fluid when incised.

Laboratory results:
None.

Microscopic description:
Liver: The submitted mass consists of cystic spaces separating small islands of hepatic parenchyma. Cystic spaces vary from 300 – 3,500 µm. Short trabeculae projecting into some lumina. Cysts are lined by low simple cuboidal epithelium encompassed by a variable amount of fibrovascular tissue. Cysts contain amorphous lightly eosinophilic material and scant histiocytes-macrophages. Bile-like pigment is not identified. Epithelium that is indistinguishable from cyst lining interdigitates with islands of hepatic parenchyma, some in branching configurations. Persisting hepatic plates are orderly. Portal triads are present but sparse and mildly disordered. Hepatocytes contain a small amount of brown-yellow granular pigment. Small islands of hematopoietic cells are throughout. There is expansion of some fibrous septa by edema, fibrin effusion, and hemorrhage.

Contributor's morphologic diagnosis:
Liver: Bile duct hamartoma (ductal plate malformation; von Meyenburg complex).

Contributor's comment:
Biliary duct hamartomas (BDH) are well recognized in human medicine. They must be distinguished from neoplastic or parasitic masses and from granulomas when detected by noninvasive imaging or exploratory surgery. Most, in the form of von Meyenburg complexes, are biologically innocent. In one survey of liver lesions in 2,843 autopsies, von Meyenburg complexes were found in 5.6% of adults and in 0.9% of children.[8] They occur as solitary or, more commonly, multiple masses. These

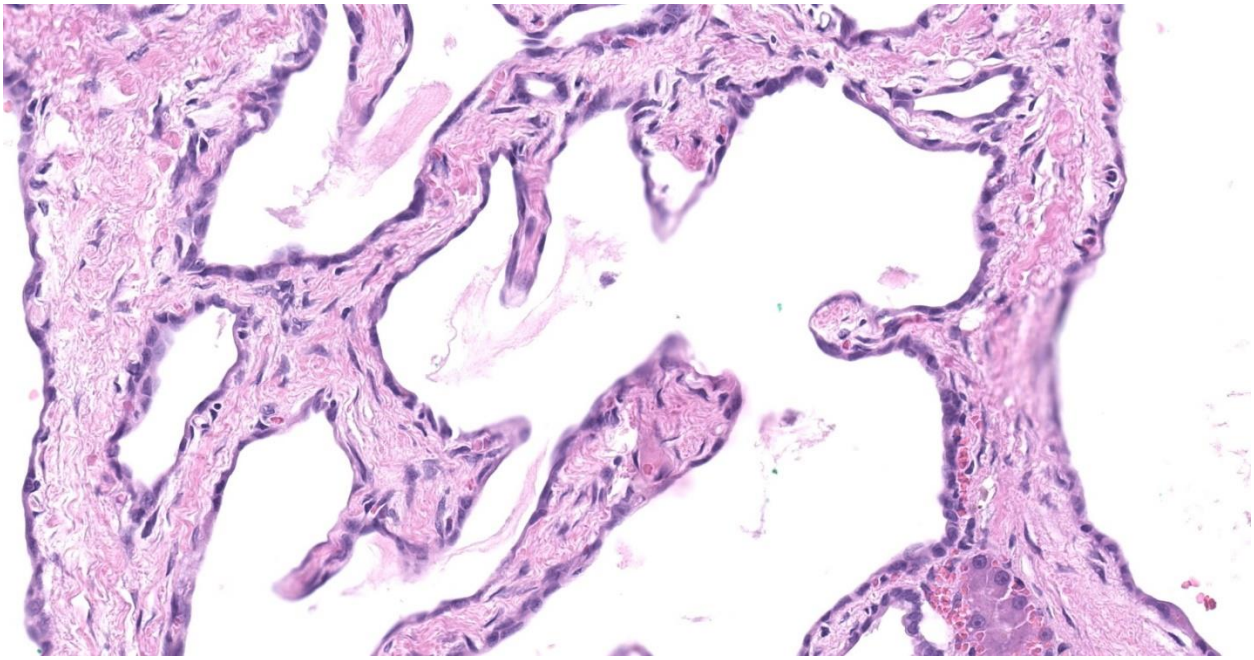

Figure 4-2. ***Liver, cat. Ectatic bile ducts are separated by dense fibrous stroma which often contains islands of entrapped hepatocytes. (HE, 62X)***

malformations arise from the ductal plate which in normal development forms intrahepatic bile ducts.[3] BDH are considered hamartomatous (from Greek: *to err*). In normal fetal ontogeny there is substantial pruning of the biliary tree as ducts and ductules develop. Typically what persist are redundant portions of the biliary tree.[4] With the advent of noninvasive imaging such as ultrasound, CT and MRI they can be recognized throughout the liver.[6] Failure to involute results in intrahepatic embryological remnants that persist into postnatal life. Some undergo cystic enlargement. A small proportion cause abdominal discomfort and may be mistaken for cholangitis.[6] As with other developmental disorders of the biliary tree, a small fraction of BDH may be precancerous.[8]

Congenital cystic disorders of the liver remain a complex and difficult entity in veterinary pathology. A current classification for congenital cystic disease of the liver in animals proposes three entities: congenital dilation of large and segmental bile ducts (morphologically similar to Caroli's disease); juvenile polycystic disease/congenital hepatic fibrosis; and adult polycystic disease, including Von Meyenburg complexes.[2]

To this submitter's eye, the mass from this cat's liver most closely approximates a von Meyenburg complex as defined in human pathology.[3,7,8] In people these complexes are generally clinically silent, as appeared to be the case here. Histological features are the presence of a variable number of dilated bile ducts embedded in fibrous stroma, which may be hyalinized.[3] These are lined by simple cuboidal to low columnar epithelium encompassing ectactic ramifying lumina.[7] They contain either eosinophilic proteinaceous material, as here, or bile-like pigment. This mass was >5 cm, which is large relative to most Meyenburg complexes found in human patients (1 – 15 mm).[4] Differential possibilities include biliary adenoma and a Caroli-like disease. Biliary adenoma is unlikely as these lack islands of hepatocytes interspersed between tubules.[2] Caroli-like disease is associated with extensive liver involvement (typically the whole organ, resulting in hepatomegaly), large duct formation, and clinical signs due to secondary bacterial cholangitis. Such features were absent here.

There are few reports of ductal plate malformations in feline species. A Caroli-type ductal plate malformation was reported in a kitten that also had a portosystemic shunt.[5] Biliary cysts

tentatively diagnosed as Von Meyenburg complexes are reported in lions.[1]

Contributing Institution:
http://www.uwyo.edu/wyovet/

JPC diagnosis:
Liver: Ductal plate malformation, fibrocystic (von Meyenburg's complex / biliary hamartoma).

JPC comment: There was energetic debate amongst participants in regard to the nomenclature of the morphologic diagnosis due to overlapping features of congential hepatic fibrocystic diseases, which in veterinary medicine have been proposed to be classified as adult polycystic disease (including von Meyenburg complexes), juvenile polycystic disease/congenital hepatic fibrosis, and congenital dilation of the large and segmental bile ducts (resembling Caroli disease in humans).[2]

Prior knowledge of the embryologic steps involved in the development of the biliary tree is beneficial in regard to understanding the pathogenesis von Meyenburg's complex and other ductal plate malformations. The liver's characteristic hepatic cords arise as a result of the early precursor cells of the liver (hepatoblasts) interacting with the capillary plexus of the vitelline veins.[3] Hepatoblasts in contact with mesenchyme surrounding large future portal vein branches become smaller, forming the primitive ductal plate.[3] These cells duplicate, forming a second layer, resulting in one layer in contact with the peripheral hepatoblasts and the second in contact with the mesenchyme surrounding the portal vein.[3] These epithelial cells become the lobular and portal layers of the ductal plate, respectively, and are separated by a slitlike lumen.[2] Smaller branches of the portal vein are subsequently surrounded as the process repeats over the following weeks.[3]

The hepatic plate then undergoes remodeling, beginning with a dilation of short segments of the double-layered ductal plates, forming tubular dilations that later detach from the lobular layer of the ductal plate due to infiltrating mesenchyme, forming primitive bile ducts.[3] Not all tubular dilations result in the formation of bile ducts, as excessive components gradually disappear, presumably by apoptosis, suggesting remodeling is a selective process.[3]

Ductal plate malformations arise due to disruption of the highly complex and epithelial-mesenchymal interactions involved in their development and subsequent remodeling.[3] Hepatic fibrosis and cysts have been identified in a significant proportion of domestic felines with concurrent polycystic kidney disease, suggesting a similar pathogenesis of each lesion's development due to an inherited autosomal dominant mutation of the gene responsible for encoding polycystin 1 (PKD1) that closely resembles the adult form of polycystic kidney disease in humans.[2] However, the association of these two lesions was not commonly observed in a study of 90 necropsied non-domestic felids.[1] Lions were found to be six times more likely to have cystic biliary lesions than other nondomestic felids; however, none of the seven affected lions were found to have concurrent renal cysts.[1] Of the 14 non-domestic felines necropsied, only one of had a concurrent renal cyst (a cougar).[1]

References:

1. Bernard JM, Newkirk KM, McRee AE, Whittemore JC, Ramsay EC. Hepatic lesions in 90 captive nondomestic felids presented for autopsy. *Vet Pathol*. 2015;52:369-376.
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; 2016, p. 264-265, 348.
3. Desmet VJ. Ludwig symposium on biliary disorders--part I. Pathogenesis of ductal plate abnormalities. *Mayo Clin Proc*. 1998;73:80-89.
4. Lev-Toaff AS, Bach AM, Wechsler RJ, et al. The radiologic and pathologic spectrum of biliary hamartomas. *Am J Roentgenol* 1995;165:309–313.
5. Roberts ML, Rine S, Lam A. Caroli's-type ductal plate malformation and a portosystemic shunt in a 4-month-old kitten. *J Feline Med Surg.* 2018;4(2):2055116918812329. Published 2018 Nov 20. doi:10.1177/2055116918812329
6. Sinakos E, Papalavrentios L, Chourmouzi,D et al. The clinical presentation of Von

Meyenburg complexes. *Hippokratia*. 2011;15:170–173.
7. Thommesen N. Biliary hamartomas (von Meyenburg complexes) in liver needle biopsies. *Acta Pathol Microbiol Scand A*. 1978;86:93-99..
8. Zimmermann A, Benign epithelial tumors and hamartomas of the biliary tract. *Tumors and Tumor-Like Lesions of the Hepatobiliary Tract.* Springer International Publishing Switzerland. 259-266. 2017