Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2022-2023

Conference #13 14 December 2022

CASE I:

Signalment:
Two, 8 month old female entire Boer goats (*Capra hircus)*

History:
A herd of nine goats were kept with a flock of sheep in a paddock associated with a school agricultural program. The goats gained access to an adjacent paddock of shrubbed land through a hole in the fence which the sheep did not find, and had been foraging in this adjacent paddock for approximately one week. Two goats were found dead initially, and a further two became acutely lethargic and depressed, and died early the following day (these two were submitted for necropsy). Within 24 hours, a further three goats had died, leaving one remaining goat from the herd that was also exhibiting acute clinical signs. One dead goat was found underneath a *Trema tomentosa* (poison peach) tree, and a further two that were observed beneath the *Trema tomentosa* subsequently died. A video was submitted of an affected goat, which showed a standing goat with a wide-based stance, salivation/foam around the mouth, and twitching of the upper lip and eyelids, with apparent unawareness of the surroundings.

Plants within the shrubbed area accessed by the goats included *Trema tomentosa* (Poison peach), *Acacia fimbriata* (Brisbane wattle), *Solanum mauritianum* (Wild tobacco), *Schinus terebinthifolius* (Broad-leaf pepperina), and an unknown species of wattle, (*Acacia* sp.) as identified by a botanist with the Queensland Herbarium.

Gross Pathology:
In one goat, the liver had a generalized, exaggerated reticular pattern, interpreted as periacinar necrosis. The same goat also had a mild pleural effusion and petechial hemorrhages in the subcutaneous tissues of the ventral neck and serosal surfaces of the rumen and small intestine. The second goat appeared slightly more autolyzed than the first, and had no grossly observed pathology. The rumen of both goats contained fragments of foliage from multiple plants species.

Figure 1-1. Presentation, goat. The affected individual demonstrated facial twitching and hypersalivation. (Photo courtesy of: The School of Veterinary Science, The University of Queensland, Gatton Campus, Queensland, Australia 4343, https://veterinary-services.lab.uq.edu.au/).

Laboratory Results:
No findings reported.

Microscopic Description:
Liver: Diffusely, there is submassive to massive necrosis spanning periacinar, midzonal and frequently periportal regions. Necrosis is characterized by disruption and loss of normal hepatic sinusoidal architecture; shrunken, fragmented, hypereosinophilic hepatocytes with pyknotic, karyorrhectic or karyolytic nuclei; or complete loss of hepatocytes, replaced by extravasated erythrocytes (hemorrhage) and small amounts of cellular and karyorrhectic debris. Multifocal intact hepatocytes in periportal areas are swollen with vacuolated pale cytoplasm (degeneration), and rare limiting plate hepatocytes are normal.

Contributor's Morphologic Diagnoses:
Liver: Severe acute submassive to massive hepatic necrosis

Contributor's Comment:
Trema tomentosa, formerly known as *Trema aspera*, and colloquially known as poison peach, is an evergreen shrub to small tree of the family Ulmaceae, which is characterised by alternately attached leaves that are elliptical- to spearhead-shaped and dark green, with finely-toothed edges and hairy upper surfaces; small clusters of greenish-white flowers in the leaf-stem junctions; and round, fleshy, black, 3-4 mm berries.[7] Poison peach is located on the east and north coasts of Australia, in the states of Victoria, New South Wales, Queensland, Northern Territory and the north coast of Western Australia.[7]

Figure 1-3. Leaves and fruit of poison peach (Trema tormentosa). (Photo courtesy of: The School of Veterinary Science, The University of Queensland, Gatton Campus, Queensland, Australia 4343, https://veterinary-services.lab.uq.edu.au/).

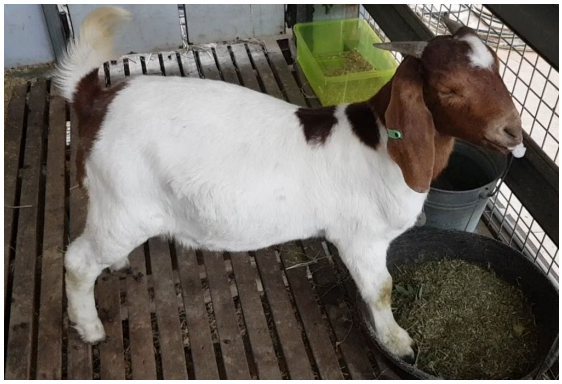

Figure 1-2. Presentation, goat. The affected individual demonstrated a wide-based stance, and its tail was curled over its back. (Photo courtesy of: The School of Veterinary Science, The University of Queensland, Gatton Campus, Queensland, Australia 4343, https://veterinary-services.lab.uq.edu.au/).

The leaves of poison peach contain a toxin known as trematoxin, which is an uncharacterised hepatotoxic glycoside, and is reported to cause fatal poisoning in cattle, sheep, goats, horses, and camels.[4,6,9,10] Clinical signs of trematoxicosis can include recumbency, anorexia, muscle fasciculations, rigid posture, reluctance to move, dyspnoea, rapid weak pulse, and salivation.[5,8,10] On post mortem examination, all reported cases have hepatic necrosis as the main feature, characterised as coagulative, periacinar to massive, and frequently with extensive hemorrhage.[5,7,8,10] Further pathology described in

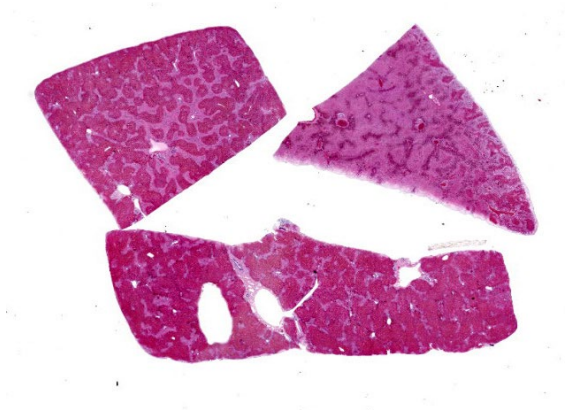

Figure 1-4. Liver, goat. Three sections of liver are submitted for examination. At subgross magnification, there is centrilobular to massive hepatic necrosis, depending on the section. (HE, 7X)

the literature is predominately hemorrhage, including subcutaneous hemorrhages of the neck, chest and abdomen; petechial hemorrhages on the serosa of the rumen, abomasum and duodenum; and subepicardial and endocardial hemorrhages.[6,8]

Another species of the same genus, *Trema micrantha*, is located in the tropical to subtropical Americas, particularly South and Central America, and Florida in North America; and is reported to cause a similar disease in horses and goats.[1,3] Additionally, there is evidence that ingestion of *Trema micrantha* by sheep can cause a fatal pneumotoxicosis, with the documented pathology including mediastinal emphysema, interalveolar septal thickening, and diffuse type II pneumocyte hyperplasia.[11]

As trematoxin is uncharacterized, definitive diagnosis of toxicity is difficult. Diagnosis is generally based on known exposure to the plant in combination with acute periacinar to massive hepatic necrosis. Differential diagnoses for acute hepatotoxicity in goats include amatoxins, cyanobacteria, *Xanthium* spp, green cestrum, cycads, gossypol, iron, polycyclic aromatic hydrocarbons, and carbon tetrachloride.[4,7] Further, intoxication with *Xanthium*, green cestrum and *Trema* spp. cause very similar gross and microscopic changes, and therefore differentiating the three syndromes may involve isolation of the specific toxin where possible, or demonstration of ingestion of the plant species.[3]

In this case, no poison peach leaves or other toxic plant leaves were identified in the rumens of the two goats necropsied. It is hypothesized by the authors and submitting veterinarian that as the leaves are fine and palatable, they were well masticated and digested quickly, or were unidentifiable within the other ingesta of the rumen. There was no known exposure to other hepatotoxins aside from poison peach, and thorough examination of the pasture by the farm hand and submitting veterinarian did not reveal further hepatotoxic plants. Additionally, the sheep that were housed with the goats were not affected. Therefore, it was concluded that ingestion of poison peach was the most likely cause of hepatic necrosis and death in this herd of goats.

Contributing Institution:
The School of Veterinary Science
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JPC Diagnosis:
Liver: Necrosis, massive.

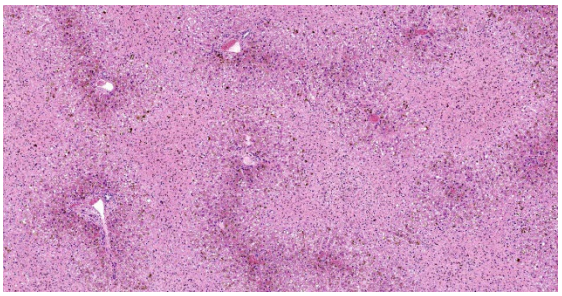

Figure 1-5. Liver, goat. There is necrosis of centrilobular and midzonal hepatocytes characterized by loss of differential staining. (HE, 76X)

JPC Comment:
This case is a classic example of centrilobular hepatocellular necrosis. Centrilobular hepatocytes are particularly susceptible to toxic injury due to their high concentration of cytochrome P450 (CYP450) and susceptibility to hypoxia due to the location furthest downstream of the hepatic artery. [3] During phase I biotransformation and detoxification, CYP450 metabolism of toxins may cause formation of reactive intermediates with local toxic effects.[3] Other examples of toxins which cause centrilobular necrosis are acetaminophen, microcystin, amanita, and *Xanthium* spp.[3] Other less common patterns of hepatocellular necrosis are periportal hepatocellular necrosis, caused by direct acting toxins like yellow phosphorous, and midzonal necrosis, which is the least common.[3]

This week's moderate, Dr. John Cullen, described the variability in histologic appearance between the different sections of liver on the slide. One possible explanation is a phenomenon called portal streaming, where blood from a specific portion of the gastrointestinal tract may be routed to specific areas of the liver through laminar flow, thus delivering different amounts of pathogens (whether toxic, infectious, or neoplastic). Other possible explanations include the different volume of each load, some regulation of flow which redirects blood flow, or sampling technique. Dr. Cullen explained that similar variation between lobes is seen in copper deposition and this variation demonstrates why sampling all hepatic lobes is critical for accurate surgical biopsies.

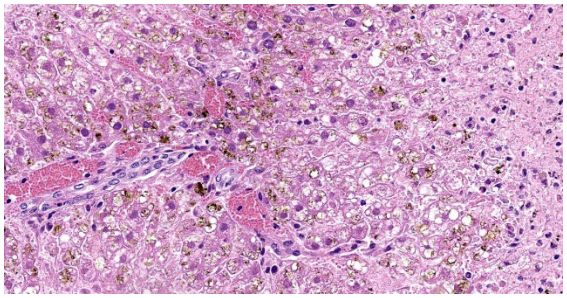

Figure 1-6. Liver, goat. Periportal hepatocytes (left) are degenerate, characterized by cellular swelling and lipidosis. There is abundant brown pigment within the cytoplasm of hepatocytes and Kupffer cells. (HE, 381X)

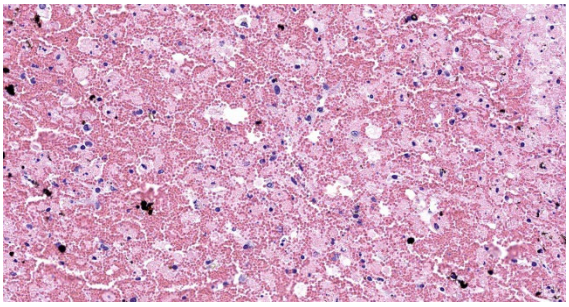

Figure 1-7. Liver, goat. Areas of hepatocellular necrosis demonstrate hemorrhage and stomal collapse. (HE, 381X)

The contributor described a related plant, *Trema micrantha* (Jamaican nettletree or capulin), which causes centrilobular necrosis in horses, goats, and sheep, and also causes pneumotoxicity in sheep. Ingestion of the plant, which appears to be highly palatable, can also cause acute neurotoxicosis without hepatotoxicosis in horses.[2,6] A recent report described *T. micrantha* ingestion by 14 horses in Brazil with primary neurotoxicosis.[6] Horses ingested the plant after being food restricted or having access to fresh trimmings and were ataxic with progressive neurologic signs resulting in death within one week. [6] On necropsy, the brains were diffusely yellow with scattered foci of hemorrhage and necrosis, and the pons most severely affected. [6] Three horses had similar spinal cord lesions. On histology, there was multifocal fibrinoid necrosis of vessels, thrombosis, and liquefactive necrosis in the brainstem, cerebellum, and, less frequently, in the spinal cord. [6] Centrilobular hepatocellular necrosis was present in only five cases and was characterized as mild. [6] Horses with *T. micrantha* toxicity may also develop neurologic signs secondary to hepatotoxicity and hepatic encephalopathy.[6] Histologically, these cases feature perivascular edema and Alzheimer type II astrocytes.[2,6] These were not observed in the recent report, and the hepatic lesions were mild to absent, so hepatic

encephalopathy was not suspected in these horses.[6] The authors speculate that there may be intermediate metabolites which have species-specific toxic effects.[6]

References:

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CASE II:

Signalment:
20-month-old, female Droughtmaster, ox (*Bos Taurus x indicus*)

History:
Inappetence and weakness; pale mucous membranes; black, watery feces and reduced gut sounds.

Gross Pathology:
Jaundice. Subcutaneous and interstitial pulmonary edema. Pale and yellow liver, marked distention of the gall bladder with mucoid bile.

Laboratory Results:
No findings reported.

Microscopic Description:
There is diffuse hepatocellular disassociation. Hepatocytes are often enlarged and many are multinucleated, containing 2-4 nuclei. The cytoplasm of hepatocytes if often distended with fine vacuolation. Multifocally and often in centrilobular areas, there is mild

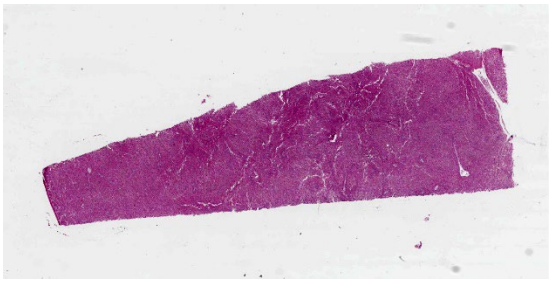

Figure 2-1. Liver, ox. A single section of liver is submitted for examination. There is a homogeneity to the section at subgross magnification resulting from loss of hepatocellular plate architecture. (HE 8X)

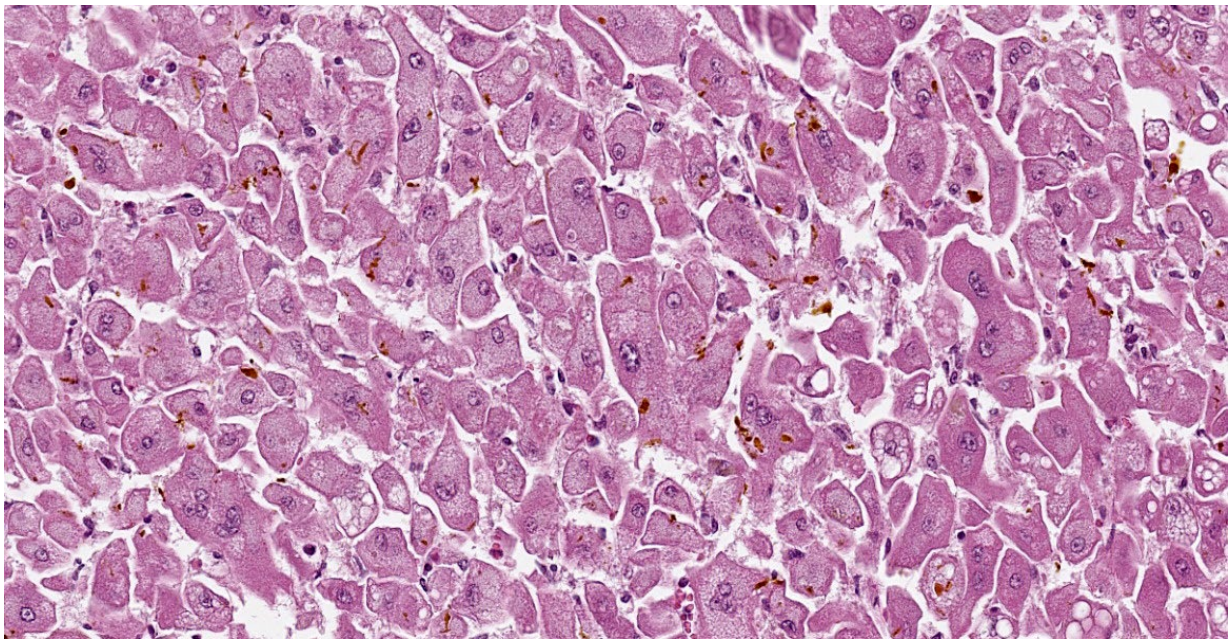

Figure 2-2. Liver, ox. Hepatocytes are diffusely swollen, vacuolated, individualized, and there is loss of normal sinusoidal architecture. There is canalicular cholestasis. (HE, 300X)

to moderate distension of bile canaliculi with bile, this is also present within the cytoplasm of hepatocytes. Multifocally, there is moderate biliary ductule hyperplasia. Multifocally, there is a mild, periportal population of lymphocytes and plasma cells with mild associated fibroplasia.

Contributor's Morphologic Diagnoses:
Liver: Hepatopathy with hepatocellular dissociation, cytoplasmic vacuolation and megalocyte formation; cholestasis and biliary hyperplasia.
Cholangiohepatitis, chronic, mild

Contributor's Comment:
These findings are consistent with lantana toxicity. Lantana (family Verbenaceae) is an ornamental shrub that grows to a height of 2-3 meters and has red, pink or white flowers. It is native to the tropical and subtropical areas of Central and South America and is considered a pest in many parts of the world. The family contains species such as *L. camara, L. indica, L. crenulata, L. trifolia, L. lilacina, L. involuerata* and *L. sellowiance* however *L. camara* is the most widespread and of greatest toxicity to livestock.[6] Its toxicity was first reported in Australia in 1910, this has subsequently been reported in many other countries. Livestock that are familiar with lantana rarely consume it voluntarily, toxicity mostly occurs in times of drought or when feed is otherwise scarce or in animals that have been brought in from lantana free areas.[6] Cattle are most commonly affected; it is rarely noted in sheep and goats as they are less likely to eat the plant.[7]

The significant toxins in lantana are triterpene acids, lantadene A (rehannic acid), lantadene B and their reduced forms; lantadene A appears to be the most toxic of these. The toxic dose depends on the toxin content of the species of lantana.[7] Clinical signs include jaundice, photosensitisation, inappetence and depression, ruminal stasis, constipation or diarrhoea with black, fluid faeces, dehydration and polyuria.[1,6,7] Cattle ingesting a large dose of the toxin can die within 2 days however most cases are more chronic with clinical signs being evident for 2 weeks before death.[7] The rumen can act as a toxin reservoir even after access to the lantana is prevented. Natural or experimental exposure to lantana results in increased bilirubin and globulins,

there can be either a decrease or an increase in PCV. Decreased albumin and increased gamma glutamyl transpeptidase, sorbital dehydrogenase and arginase levels have been variably noted.[1,6]

Gross findings include jaundice; an enlarged, pale and yellow/orange or green-grey liver and a gall bladder that is distended, sometimes with mucoid bile; enlarged, mottled, wet kidneys and blood-stained fluid in the abomasum and small intestine.[7] Histological findings include enlarged hepatocytes and fine hepatocellular cytoplasmic vacuolation which can be more evident in periportal areas and bile accumulation which is often more pronounced in periacinar zones. There is also often bile duct proliferation and, in some cases, periportal apoptosis or necrosis of hepatocytes.[7] The gall bladder can demonstrate mucoid metaplasia of the epithelium with hemorrhage, ulceration, inflammation and necrosis.[1] The kidneys often demonstrate mild to severe vacuolar change or necrosis of the tubular epithelium with extensive tubular cast formation.[1,7] There can also be ulceration of the abomasal mucosa.[1]

The toxin is thought to cause collapse, distension or microvilli damage in bile canaliculi. The mechanism of cholestasis is still unknown however damage to the contractile pericanalicular cytoskeleton or the cell adhesion molecules is possible.[7] The role of hyperbilirubinaemia in the nephropathy is unknown. Myocardial necrosis has been noted in sheep and may be responsible for death in animals that die early in the course of the disease.[7]

Contributing Institution:
Dr Mirrim Kelly-Bosma, School of Veterinary Science, University of Queensland, Gatton campus, Gatton, Queensland, Australia, 4343. (https://veterinary-science.uq.edu.au/)

JPC Diagnosis:
Liver: Hepatocellular dissociation, megalocytosis, multinucleation, rarefaction, lipidosis, diffuse, severe, with ductular reaction, and canalicular cholestasis.

JPC Comment:
This case illustrates a different pattern of hepatotoxicity compared to the centrilobular

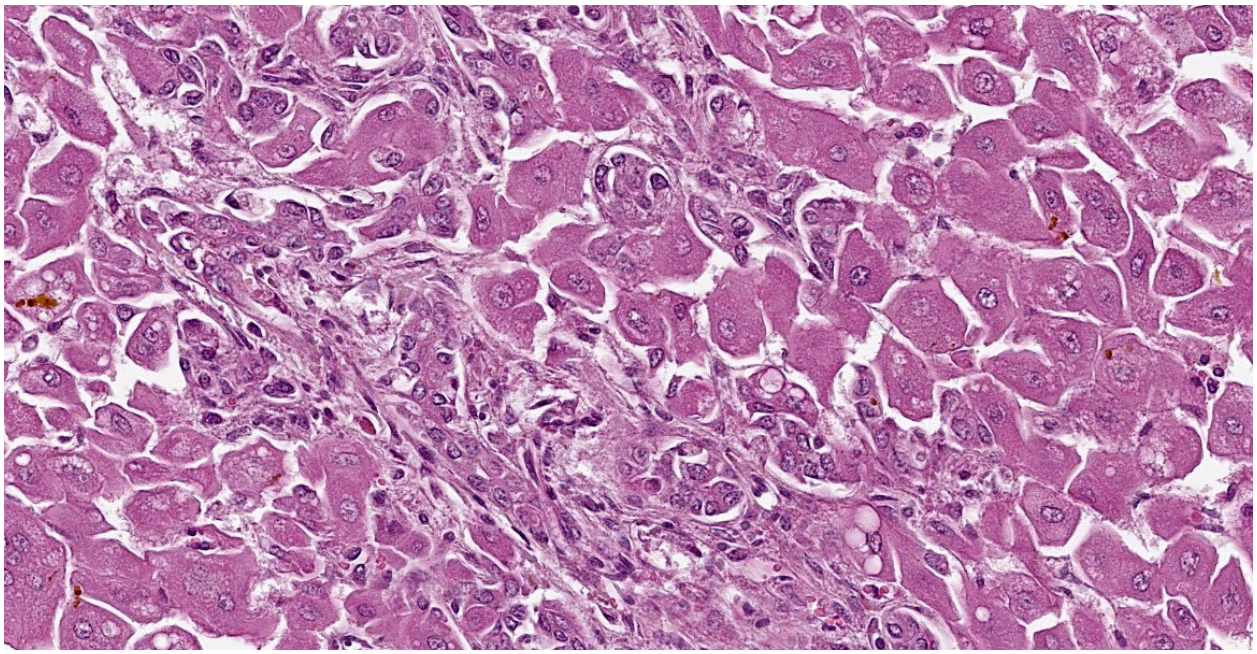

Figure 2-3. Liver, ox. There is portal fibrosis, biliary hyperplasia, and mild lymphoplasmacytic inflammation. (HE, 300X)

necrosis in Case 1 of this conference. In addition to the mechanisms of toxicity mentioned by the contributor, studies in rats have suggested that metabolites inhibit mitochondrial respiration and that reduced lantadene A may be more toxic than lantadene A.[4] The toxin targets the bile canalicular plasma membrane, causing impaired hepatobiliary excretion and intrahepatic cholestasis. The toxins inhibit neural impulses and ruminal stasis.[3,4] Hepatocellular megalocytosis is speculated to be due to enlargement of the rough endoplasmic reticulum.

Key histologic findings, as covered by the contributor, include hepatocellular megalocytosis with cytoplasmic vacuolization and canalicular cholestasis. Dr. Cullen also remarked on the pronounced individualization of hepatocytes, a phenomenon which is also seen in leptospiral infections.

Other hepatotoxins which cause megalocytosis are pyrrolizidine alkaloids and aflatoxin.[2] Repeated exposure to pyrrolizidine alkaloids causes megalocytosis with hepatic atrophy, while ingestion of large quantities causes acute centrilobular necrosis.[2] Similarly, prolonged exposure to low levels of aflatoxin can cause megalocytosis during with scattered necrosis.[2] Acute aflatoxin toxicosis in large animals has only been documented in experimental settings because there are insufficient quantities in moldy feed to induce acute injury.

Another key clinical and histologic feature for lantana toxicosis is cholestasis, and in herbivores, cholestasis can quickly lead to type III (hepatogenous) photosensitization due to the lack of biliary excretion of chlorophyll breakdown products (phylloerythrins) in the blood.[2] When deposited in the skin and exposed to UV light these pigments cause reactive oxygen species formation and tissue damage.[5] Hepatogenous photosensitization is usually caused by mycotoxins and hepatotoxic plants, including sporidesmin and lantana, although it has been associated with a wide range of plants, including normal forage crops like Bermuda grass.[5] Grossly, photosensitization manifests as erythema, edema, and necrosis in sparsely haired or unpigmented skin, and affected animals are pruritic. Histologically, there is edema of the dermis and coagulative necrosis and vesicles in the epidermis.[5]

References:

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CASE III:

Signalment:
The canine puppies are a mixture of German shepherd, Alaskan malamute, and Norwegian elkhound. It is unknown how large the litters were, but all puppies had clinically healthy littermates. The breeder also reports that a single puppy from a previous litter (also with the same parentage), died at four weeks old.

History:
Three female mixed-breed canine puppies from two sequential litters with the same parents, died at home at less than 5 weeks of age. All animals had a history of poor weight gain, and pale grey, waxy, odorless feces. Puppy #1 (from litter "A") was approximately 3 weeks old and weighed 555 g. Puppy #2 and #3 (both from litter "B") were both approximately 4 weeks old and weighed 1235 g and 2000 g, respectively.

Gross Pathology:
All puppies were in thin body condition, with reduced internal fat stores. The distal small intestine and colon contained thick, pale grey-tan digesta (interpreted as acholic). Puppies #2 and #3 were also noted to be mildly icteric. There was no evidence of extrahepatic portal shunt vessels, and the external anatomy of the livers was normal. In all puppies, at the location of the expected gall bladder and common bile duct, there was a firm tubular structure with no bile content on cut section. It was not recorded in the gross post mortem reports for puppy #1 or #2 whether the major duodenal papilla was present, but the opening of the major duodenal papilla was not grossly appreciable in puppy #3. Puppy #1 had dark black mucous over the rugal folds of the stomach, and similar dark tarry content in some areas of the small intestine and within the colon.

Laboratory Results:
No findings reported.

Microscopic Description:
There is abnormal liver architecture, with no appreciable portal triads, as no biliary epithelium was evident. Portal veins were irregularly spaced, and frequently accompanied by small, reduplicated arterioles, often oriented along the limiting plate and occasionally branching out within the hepatic parenchyma. Hepatocytes were arranged in disorganized cords up to three hepatocytes thick, and occasionally in a more sheet-like formation. Where present, sinusoids were often dilated or ectatic, and adjacent hepatocytes

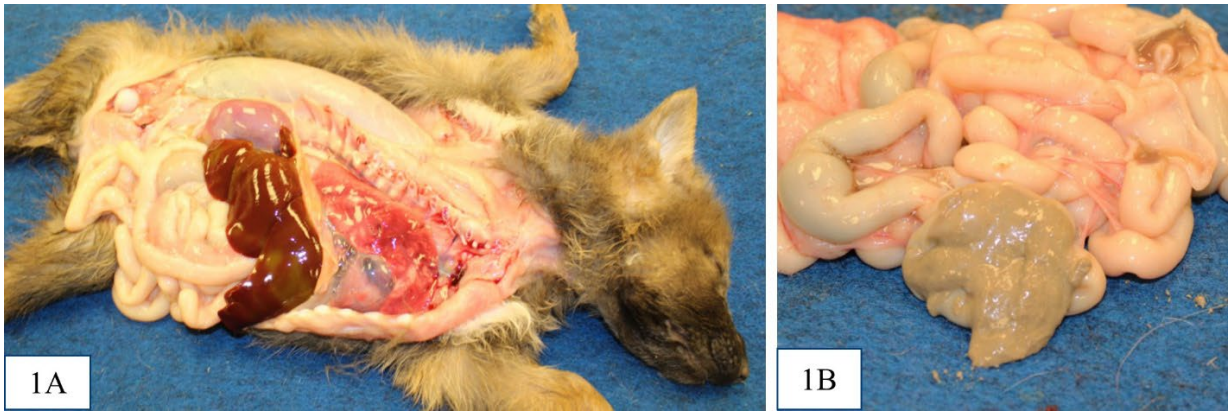

Figure 3-1. Presentation, dog. (From Puppy #3) A) On post mortem external assessment, all animals were in poor body condition. B) The distal small intestine and colon contained light grey-tan digesta (interpreted as acholic and confirmed using Hall's bile stain). (Photo courtesy of: Ontario Veterinary College, Department of Pathobiology, Guelph https://ovc.uoguelph.ca/pathobiology/)

were thin and atrophic. There were innumerable bile plugs in canaliculi throughout the parenchyma. Frequently, Kupffer cells and up to 30% of hepatocytes contained scant fine brown granular pigment.

Contributor's Morphologic Diagnoses:
Congenital atresia of the biliary tract and intrahepatic cholestasis.

Contributor's Comment:
The three puppies had absence of the gall bladder and intrahepatic bile ducts, with intrahepatic cholestasis. The lack of CK7 positive cells forming distinct tubules throughout the liver confirms the diagnosis of complete absence of intrahepatic bile ducts.

In broad categories, the absence of intrahepatic bile ducts and a gall bladder could be because they never formed or that they had partially or completely formed, and then were later lost. During organogenesis of the liver and biliary system, a part of the endoderm forms the hepatic diverticulum, which subdivides into a cranial portion (pars hepatica) and a caudal portion (pars cystica).[4] The gall bladder develops from the pars cystica, and the timing of this development and the precise pathways involved have not been well characterized.[15,23] In contrast, the common bile duct, intrahepatic biliary system, and hepatocytes differentiate from bipotential progenitor hepatoblast cells that differentiate in the pars hepatica– the hepatoblasts within the liver parenchyma become hepatocytes, and those at the interface between the parenchyma and the portal mesenchyme surrounding portal veins strongly express biliary-specific cytokeratins and become biliary epithelial cells that form a single layer referred to as the ductal plate.[3] In the development of intrahepatic bile ducts, just after day 16.5 of embryogenesis in mice when the ductal plate becomes partially bilayered, small focal dilations appear between the two cells layers, giving rise to bile ducts.[7] As the puppies in this case series lacked both a gall bladder and intrahepatic biliary structures, the deviation from normal organ development may have happened at the level of the diverticulum before the subdivision into the pars cystica and pars hepatica, or less likely, there may have been issues in both developmental pathways after the division.

There are a variety of conditions characterized by intrahepatic biliary maldevelopment in humans, which in the literature is referred to as paucity of interlobular bile ducts, intrahepatic biliary hypoplasia or aplasia, intrahepatic biliary atresia, ductular paucity, ductopenia, and ductular hypoplasia.[1,9] Complete congenital absence of the intrahepatic bile ducts has been reported in ten children, many of whom died in early childhood. Unlike these puppies, none of the human cases were reported to have an abnormal extrahepatic biliary system except for one child that had fibrotic cystic and common bile ducts (not observed in any of the canine cases).[12]

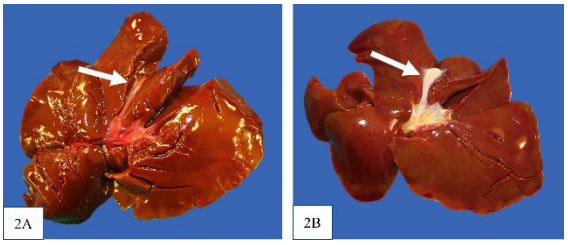

Figure 3-2. Liver, dog. All puppies had livers with unremarkable gross architecture, and no evidence of extrahepatic shunts. All three puppies also had very small, thin tubular structures in the location of the gall bladders (indicated by the arrow) that lacked bile content. A) Liver from Puppy #2. B) Liver from Puppy #3. (Photo courtesy of: Ontario Veterinary College, Department of Pathobiology, Guelph https://ovc.uoguelph.ca/pathobiology/)

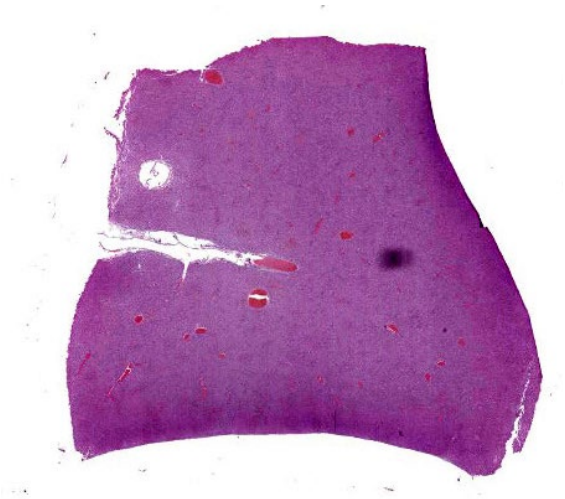

Figure 3-3. Liver, dog. A single section of liver is submitted for examination. (HE, 8X)

Extrahepatic biliary atresia has also been reported in humans, and also rarely in domestic species including dogs, lambs, cats, monkeys, calves and horses.[8,13,14,18,20,22,25] The pathogenesis suggested in all species involves progressive fibrosis resulting in disconnection of the common bile duct from the intrahepatic biliary tree, occurring anywhere from the duodenum to the porta hepatis.[14,16] Extrahepatic biliary atresia in some affected animals was considered naturally occurring, and in others, occurred following toxin exposure (e.g. experimentally induced with fetal trypan blue exposure in piglets, plant toxins in sheep and calves).[14,19] In the two reported cases in young dogs, a distended gall bladder was appreciated, with bile duct obstruction. Cholecystoduodenostomy was performed to surgically circumvent the obstruction in both cases, reportedly with a good short-term clinical outcome in one case.[25] In the other case, involving a border collie puppy, the dog died 6 weeks after the cholecystoduodenostomy was performed due to biliary and hepatic toxocariasis, and histology of the common bile duct at the site of the atresia demonstrated replaced of the bile duct by solid fibrous tissue.[22] Based on the literature, the extrahepatic biliary atresia previously reported in dogs is different from the puppies described in this case series, as there was no evidence of normal gall bladder or common bile duct structures. Additionally, extrahepatic biliary atresia in all species and humans eventually leads to increased connective tissue within portal areas and surrounding proliferating biliary ductules – as we had no intrahepatic biliary structures or increased connective tissue in our cases, the condition affecting these puppies is distinctly different.[2]

Congenital absence of a gall bladder has also been reported in dogs, most often affecting small breed dogs, including Chihuahuas (10 of 17 reported cases), toy poodles (3 of 17 reported cases) and Jack Russell terriers (1 of 17 reported cases), and most often accompanied by hypoplasia or absence of some hepatic lobes.[21] Histologically, all dogs with gall bladder agenesis had similar findings to extrahepatic biliary atresia, with biliary hyperplasia and portal fibrosis, which does not fit with our case series.[21]

Very little literature exists on gall bladder development, but there have been a few key pathways identified in intrahepatic biliary epithelium development which may be abnormal in these puppies. The most common cause of intrahepatic biliary maldevelopment in humans is Alagille syndrome, an autosomal dominant disorder with variable expressivity which is also characterized by at least three of the five main clinical manifestations, including chronic cholestasis, vertebral arch defects, pulmonary artery hypoplasia or stenosis, posterior embryotoxon, and a particular set of facial features.[10] This syndrome does not include absence of a gall bladder, and these puppies lack the other clinical manifestations of the syndrome, but knowledge of this human condition provides some insight into potential genes and signalling pathways that could be abnormal in this case series.[1]

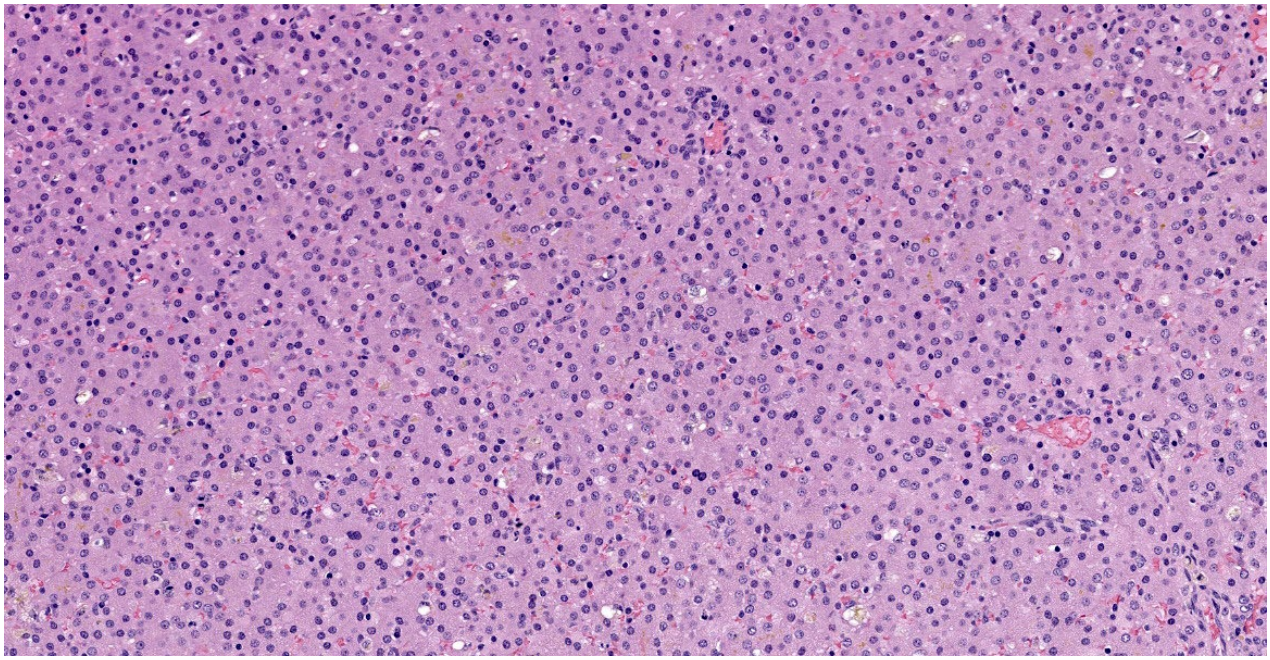

Figure 3-4. Liver, dog. There is diffuse loss of hepatic plate architecture with hepatocytes arranged in thick trabeculae or sheets, and portal areas are markedly reduced or absent. (HE, 8X)

Genetically, Alagille syndrome has been characterized by a duplication of exon 6 of the JAG1 gene on chromosome 20, which encodes a Notch1-signalling pathway with importance in embryogenesis.[26] Notch signalling has been demonstrated to be required for intrahepatic bile duct and ductal plate formation in mice, and loss of Notch signalling in mouse hepatoblast progenitor cells results in reduction of peripheral intrahepatic bile duct branches postnatally.[11,16,24,27] Research of Alagille syndrome has also led to the discovery of transcription factor protein hepatocyte nuclear factor (HNF)-6, which appears to regulate the number of cells that are stimulated to transition from a hepatoblast to a biliary epithelial cell.[7] It is suspected that the levels of this protein influence and are influenced by the hepatic mesenchyme, which also plays a vital role in the transition to biliary epithelium.[23]

Lastly, to explore the possibility that these puppies had biliary trees that had partially or completely formed, and then were later lost, drug-induced "vanishing bile duct syndrome" was briefly considered, which is a human condition that develops following toxin-induced damage to bile ducts.[9] Over thirty drugs are implicating this syndrome, which in its chronic form is clinically characterized by prolonged jaundice or chronic cholestasis for more than one year following administration of one of these drugs, and is histologically characterized by a bile to portal tract ratio of less than 0.5.[8,9] Although these dogs meet the defined criteria for "ductopenia" as described by the literature, these dogs have no known history of drug exposure, and the canine cases in this series lack portal tracts infiltrated by mononuclear cells (which is commonly seen in the human condition).[9] Lastly, again, this phenomenon has also not been reported in association with absence of a formed gall bladder.[9]

In summary, the present case report demonstrates the existence of a novel canine condition characterized by congenital absence of both intrahepatic bile ducts and the gall bladder and extrahepatic biliary tree. According to medical dictionaries, atresia is defined as "abnormal closing or absence of a tube in the body" whereas aplasia is defined as "lack of growth of tissue".[6] Using this nomenclature, arguably both "extrahepatic and intrahepatic biliary atresia" and "extrahepatic and intrahepatic biliary aplasia" could be appropriate when describing these puppies. However, considering that we were unable to unequivocally characterize the CK7 positive duct in the submucosa of the duodenum as being pancreatic or hepatopancreatic in origin, and leaving open the possibility that it may reflect a small portion of an intact common bile duct (which would imply that there is a regionally extensive absence of the extrahepatic biliary tree), "extrahepatic and intrahepatic biliary atresia" may be more appropriate in this case. As multiple litters with the same parentage were affected, we suspect an underlying genetic cause.

Contributing Institution:
Ontario Veterinary College, Department of Pathobiology, Guelph
https://ovc.uoguelph.ca/pathobiology/

JPC Diagnosis:
Liver: Biliary aplasia.

JPC Comment:
The contributor provides an excellent review of embryologic development of the liver and gallbladder as well as the existing literature on gall bladder agenesis and biliary atresia. Another novel presentation of gall bladder agenesis was recently reported by Mestrinho et al in a 1-year-old male French bulldog. In addition to gall bladder agenesis, the dog had an umbilicobiliary fistula that resulted in persistent yellow discharge from the umbilicus.[17] A duct-like structure connected the umbilicus to the common bile duct; histopathologic examination of the resected structure revealed similar features to a gall bladder or bile duct.[17] The authors speculated that the pars cystica of the hepatic diverticulum became trapped during invagination of umbilical cord structures and subsequently formed a cutaneous fistula.[17]

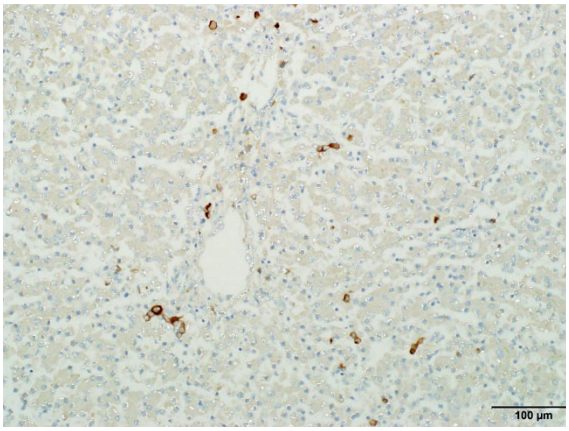

Figure 3-5. Liver, dog. In Puppy #3, immunohistochemistry for cytokeratin 7 (CK7) showed rare individual and paired cells along the limiting plate adjacent to portal veins, but no organized ductular structures. In the other two cases, there were no CK7 positive cells in the liver. (Photo courtesy of: Ontario Veterinary College, Department of Pathobiology, Guelph https://ovc.uoguelph.ca/pathobiology/)

Dr. Cullen and conference participants discussed the greater amount of bile but lesser degree of hepatocellular damage in this case compared to case 2 (lantana toxicity in a goat), in whic bile canaliculi are injured, and liberated bile salts caused damage to hepatocellular membranes. Participants elected to use "aplasia" as a morph due to the complete lack of biliary profiles; most other forms of biliary atresia which frequently feature small CK7 positive biliary profiles.

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CASE IV:

Signalment:
3-year-old, intact male, Syria Hamster, *Mesocricetus auratus*

History:
It was reported by the owner that the patient had been progressively ailing over a three-month period and that a small mass had been present on his right eye for at least 6 months. On October 14th, 2020, he was found to be cold, trembling, and weak for several hours, but aware of his surroundings. The slight trembling of his hind limbs continued to the next day. Later, the abdominal organs became progressively enlarged and visible through his skin. Four months later the patient became lethargic and began open mouth breathing. The left eye became proptosed and unable to be closed. Patient progressed to teeth grinding and lateral recumbency, with closed mouth, but labored breathing. Patient was euthanized shortly after.

Gross Pathology:
The left lateral, left medial, and right medial liver lobes are fully to partially effaced by soft, multilobular masses that measure 1cm x 3cm x 1.5cm to 2cm x2cm x1cm. The masses are black to red, soft, fluid filled.

Laboratory Results:
No findings reported.

Microscopic Description:
Liver: In multifocal areas the hepatic parenchyma is replaced by large cystic structures lined primarily by a single layer of cuboidal epithelial cells; occasionally there are up to three layers of cells. The lining cells occasionally form variably sized papillae that project into the lumen of the cysts. The lumen of the cystic structures contains an amphophilic, homogenous, vesiculated fluid substance. Portal regions frequently are infiltrated with mild to moderate numbers of lymphocytes and plasma cells. Most portal regions have mild to marked bile duct hyperplasia. In areas of severe bile duct hyperplasia, the surrounding hepatocellular parenchyma contains multifocal areas of hemorrhage with distortion of

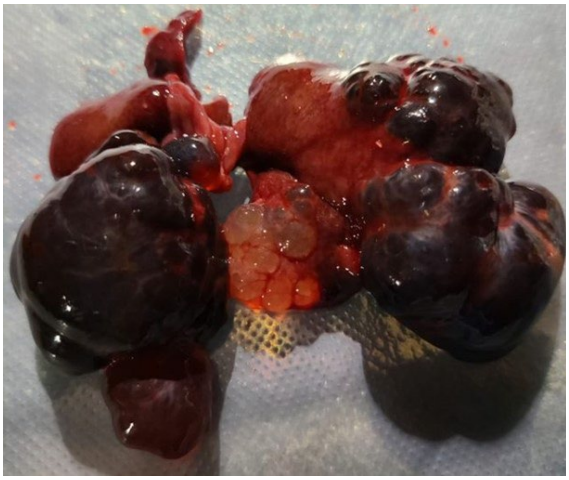

Figure 4-1. Liver, hamster. Tissue from a 3-year-old Syrian hamster. The liver is markedly enlarged and the majority of the hepatic parenchyma of all lobes has been replaced or expanded by fluid filled cysts. (Photo courtesy of: Tuskegee University, College of Veterinary Medicine, Department of Pathobiology, https://www.tuskegee.edu/programs-courses/colleges-schools/cvm)

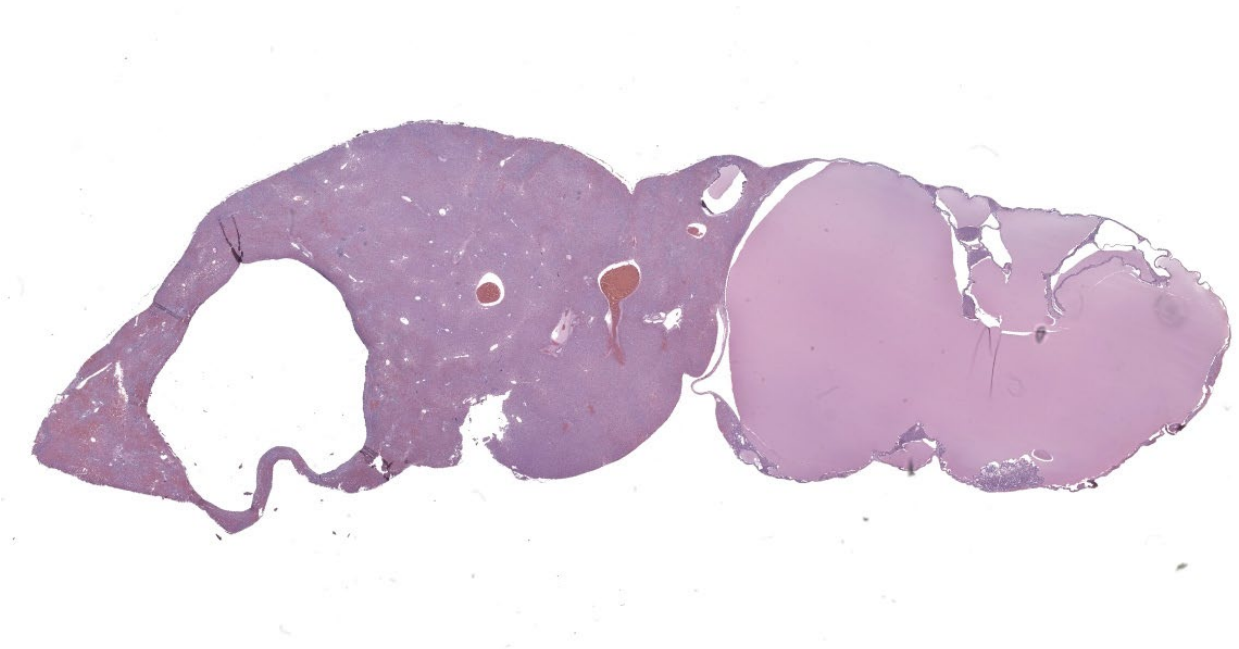

Figure 4-2. Liver, hamster. A single section of liver with multiple large, occasionally fluid filled cysts is submitted for examination. (HE, 6X)

hepatic cord architecture and individual hepatocyte necrosis.

Contributor's Morphologic Diagnoses:
Multifocal hepatic cysts (polycystic liver disease)

Multifocal moderate to marked bile duct hyperplasia and lymphoplasmacytic periportal hepatitis with multifocal hemorrhage and individual cell necrosis

Contributor's Comment:
This Syrian hamster had many of the common findings in aged hamsters including severe glomerulonephropathy, an atrial thrombus, and marked hepatic disease.[14] Although hepatic cysts are very common in older hamsters, they are thought to be the result of disconnected biliary structures present during hepatic development.[3] Ductal structures become disconnected from the biliary tree through loss or defects in genes signaling.[5] Affected animals remain asymptomatic until cyst growth initiates in adulthood.

Polycystic liver disease (PLD) is very well characterized in humans and is subdivided into three distinct entities.[3] The diseases are categorized by both the genetic mechanism and the gross and histological appearance of the lesions. The first is Von Meyenburg complexes (VMC) also called biliary hamartoma or hepatic cystic hamartoma.[9] These lesions have characteristic small, nonhereditary nodular cystic lesions which histologically appear as discrete fibrotic areas with small, irregular bile ducts. The second is isolated polycystic liver disease (autosomal dominant PLD) with presence of innumerable hepatic cysts and autosomal dominant heredity. The final category of PLD is polycystic kidney disease (PKD) where cysts are present in both the kidneys and liver.[19]

Polycystic liver disease in hamsters is characterized by multiple hepatic cysts that eventually lead to the replacement of normal liver parenchyma.[10,13] The etiology of PLD in hamsters has not been completely elucidated but much research has been done to understand PLD in isolation and PLD in association with polycystic kidney disease (PKD) in

humans. Non PKD associated PLD is the result of mutations in several genes including LRP5, PRKCSH, and SEC63 which are in charge of making the proteins sec-63 and hepatocystin.[16] These gene are also responsible for fluid transportation and epithelial cell growth. In polycystic liver disease associated with PKD, renal complications such as renal failure are more common.[16] PKD1 and PKD 2 are the genes are responsible for making polycystin 1 and polycystin 2 and mutation leads to dysregulation of fluid secretion and abnormal growth, ultimately leading to cyst formation.[4]

Hepatic cysts associated with PLD must be differentiated from cysts that might be seen in association with hepatic neoplasms in which cysts may develop such as cholangiocarcinoma or cystadenoma. Syrian hamsters, in general, have a low prevalence of spontaneous tumors of the liver.[8] Kondo et al examination of 15 Syrian hamsters found a single hepatic hemangioma.[8] The epithelial cell papillary projections, hepatic degeneration, necrosis, and leukocyte infiltration seen in this case is not typically seen in PLD but are common findings in neoplastic disease of the liver.[16] True cysts are usually multiple and lined by a single layer of epithelium, whereas tumors can be single or multiple, uni- or bilateral, and may have a complex arboriform pattern. The lack of cellular pleomorphism, stromal invasion, and mitotic figures suggest a non-malignant process. Cysts and cyst-like

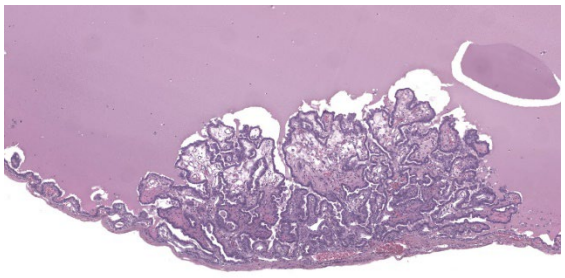

Figure 4-3. Liver, hamster. Epithelium lining the largest of the biliary cysts forms papillary and micropapillary structures into the lumen. (HE, 76X)

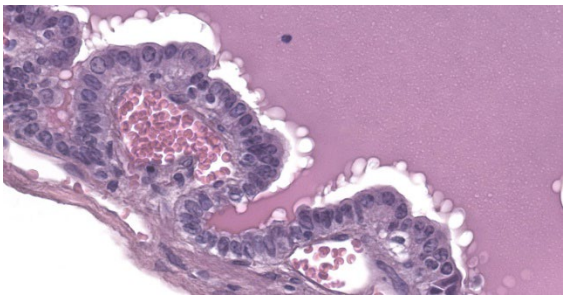

Figure 4-4. Liver, hamster. A single layer of columnar biliary epithelium lines the cysts. (HE, 7X)

structures that develop from parasites, abscesses, or hematomas must also be differentiated from PLD.[1,15]

Contributing Institution:
Tuskegee University, College of Veterinary Medicine, Department of Pathobiology, https://www.tuskegee.edu/programs-courses/colleges-schools/cvm

JPC Diagnosis:
Liver: Biliary cysts, multiple, with bridging ductular reaction and focal intraductal biliary papillary hyperplasia.

JPC Comment:
In hamsters, this relatively common incidental lesion observed in older animals may be accompanied by cysts in the epididymis, seminal vesicles, pancreas, and endometrium.[15] Infrequently, there may be clinically detectable hepatomegaly and ascites with straw colored fluid. The surrounding hepatic parenchyma typically shows pressure atrophy, necrosis, and congestion with possible hemorrhage.[18] Adjacent hepatocytes may demonstrate vacuolar change and there may be biliary ductular reaction within periportal regions.[18]

Conference participants discussed the presence of focal papillary projections into the cyst lumen, and considered the possibility that ductal plate anomalies may evolve into a neoplastic process (biliary cystadenoma), or a neoplasm may spontaneously occur in the

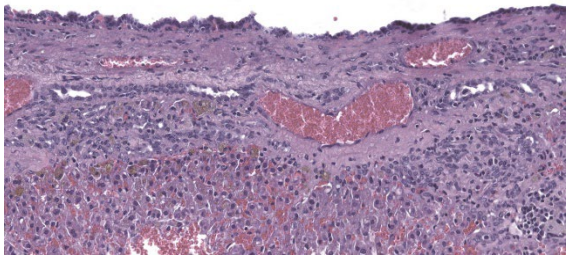

Figure 4-5. Liver, hamster. There is loss of subcapsular hepatocytes, mild biliary hyperplasia, and mild mesothelial hyperplasia. (HE, 267X)

same tissue. Dr. Cullen explained that because the genetic mechanism for polycystic liver disease has not been elucidated in this species, the specific pathogenesis creating papillary projections cannot be distinguished. Participants decided that the inflammation in the section is a secondary reactive hepatitis and elected not to include it in the morphologic diagnosis.

Polycystic liver has been documented in many species, including dogs, cats, pigs, mice, several deer species, chamois, llamas, alpacas, and various fish species.[6,7,12,18,20] Spontaneous polycystic liver has recently been documented in seven K14-LacZ transgenic mice; cysts were lined with cuboidal epithelium that was occasionally ciliated.[12] Affected males also had seminiferous tubular degeneration and a spermatogenesis, while two of the three females had ovarian cysts; affected mice appeared to be infertile.[12]

In veterinary medicine, cystic liver lesions may also be a component of adult or juvenile polycystic diseases.[2] Adult polycystic kidney disease, which has a higher prevalence in bull terriers and Persian cats, is a slowly progressive disease which may include cysts in other organs, such as the liver.[2] In humans, the disease is linked to autosomal dominant mutations in PKD1 and 2, and in dogs and cats, it has been linked to PKD1.[2,17] The mutation causes modification of the polycystin-1 protein in the primary cilia, which forms the centriole during mitosis, serves as a mechanoreceptor, and plays a role in fluid transport. Ciliary dysfunction appears to decrease fluid absorption during cyst development.[17] This disease may feature cysts in the liver and pancreas as well as hepatic fibrosis.[2] An autosomal recessive form of polycystic kidney disease in humans has been associated with a mutation causing dysfunction of fibrocystin, another component of the cilia.[2] This has been documented in West Highland white and Cairn terriers and some breeds of sheep and may also feature cysts in the liver.[2]

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