Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2019-2020

C o n f e r e n c e 7

9 October 2019

Conference Moderator:
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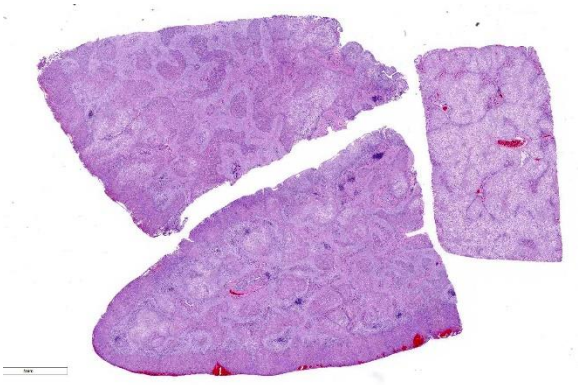
CASE I: AP19-00238-9 (JPC 4135938).

Signalment: 12-year-old female spayed domestic longhaired cat (*Felis catus*)

History: The patient presented to the NCSU Small Animal Emergency Service on 1/19/19 with a history of steady decline over the past several months with weight loss, anorexia, and vomiting. Over the last few days, the patient had also developed neurologic signs. The primary veterinarian's bloodwork found elevated liver enzymes (ALT of 166, AST of 124, ALP of 311, total bilirubin of 2.8, and conjugated bilirubin of 1.5), a mildly elevated SDMA (16, reference 0-14 ug/dl), mildly reduced BUN (15, reference 16-37), and a mildly reduced creatinine (0.8, reference 0.9-2.5). Abdominal ultrasound identified bilateral chronic nephropathy with small nephroliths or dystrophic mineralization and a splenosystemic collateral vessel. The primary veterinarian treated with a renal diet, ursodiol, and lactulose. The owners felt clinical signs did not improve. On physical exam at NCSU, she had a reduced BCS and moderate muscle wasting and was quiet and depressed with intermittent responsiveness. Due to concern for the quality of life, euthanasia was elected.



Liver, cat. Three sections of liver are submitted for examination. Approximately 50% of the hepatic parenchyma is replaced by anastomosing bands of dense fibrous connective tissue, which contain randomly scattered cellular aggregates (HE, 5X)

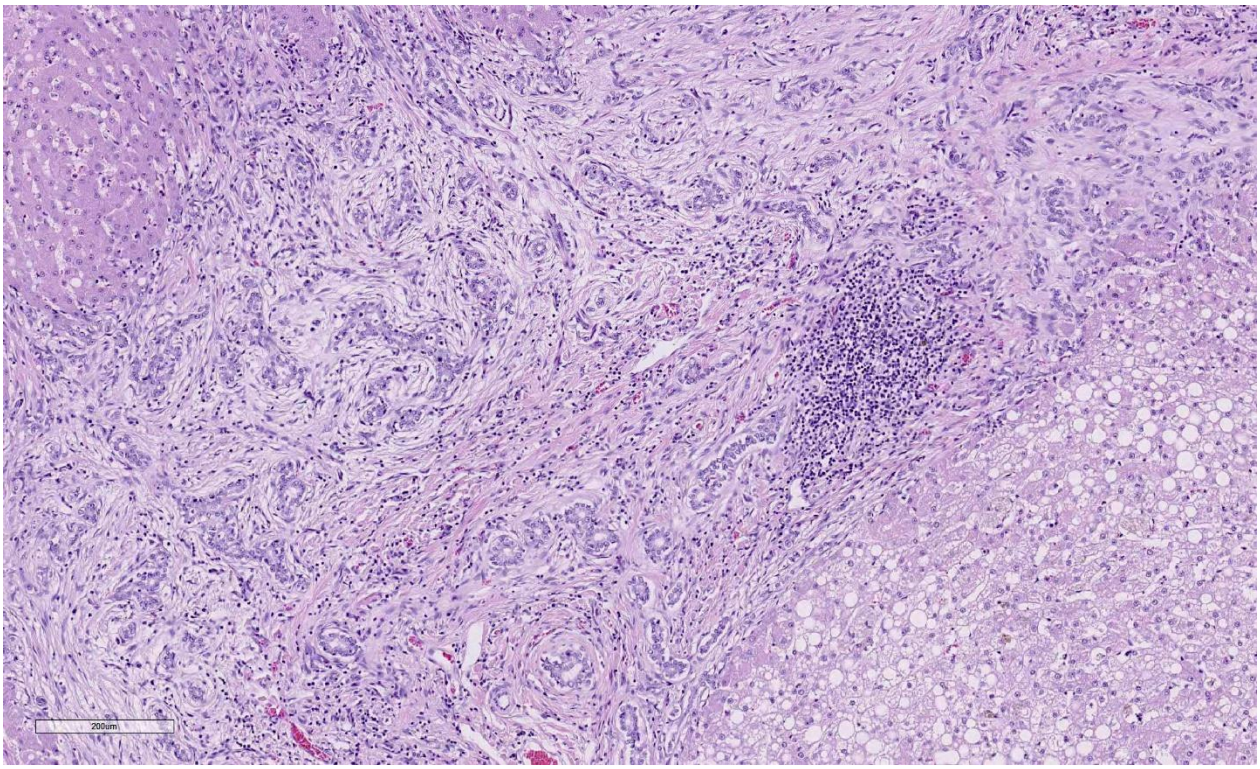
Gross Pathology: Arising from the splenic vein and connecting to the caudal vena cava

in the region of the left kidney is a large shunt vessel up to 4 mm in diameter. Additionally, connecting the portal vein and caudal vena cava in the cranial abdomen are two small (1 mm in diameter), tortuous vessels. The liver is mildly reduced in size, weighing 67 g (2.9% of total body weight; normal is 3-4). The liver is diffusely markedly pale red to tan and moderately firm. Over the diaphragmatic surface of the right lateral liver lobe is a 2.0 x 0.8 cm region of hemorrhage and mild depression. The parenchyma subjacent to this focus is markedly firm. The right kidney is mildly reduced in size compared to the left kidney and has multifocal
chronic infarcts up to 2 cm in diameter. No abnormalities are identified externally in the brain.

Laboratory results: No additional findings

Microscopic Description:

Liver: Replacing 20-50% of the hepatic parenchyma is robust, portal-to-portal bridging mature collagenous stroma, and within this stroma are markedly increased numbers of small caliber bile duct profiles. Bile ducts often have irregular to absent lumina, are tortuous with occasional branching, and frequently occur at the limiting plate directly adjacent to periportal hepatocytes. Mildly increased numbers of arteriole profiles accompany hyperplastic bile ducts. Portal veins are frequently reduced in diameter or absent. Within this bridging fibrosis are mild, multifocal infiltrates of lymphocytes and plasma cells. In the subcapsular region, sinusoids are multifocally markedly congested. The mesothelium is regionally hypertrophied. Variably 15-75% of hepatocytes, in a patchy predominantly centrilobular to midzonal distribution, contain one to multiple, small to



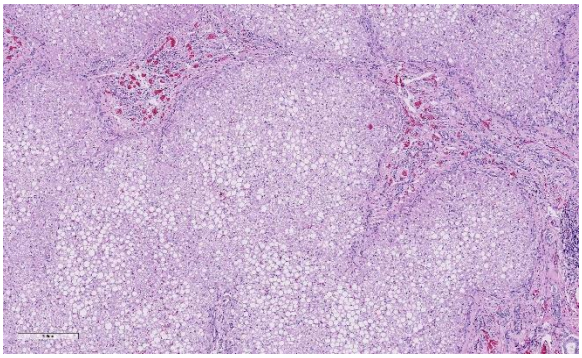
Liver, cat. Higher magnification of the dense bands of fibrous connective tissue which expands and bridges portal areas and largely effaces the periphery of the hepatocellular lobule. There is marked biliary hyperplasia in areas of fibrosis, and scattered areas of lymphoplasmacytic inflammation. (HE, 107X)

large, discrete, clear, lipid type vacuoles. Scattered within the parenchyma are occasional pigment granulomas composed of foamy macrophages that are occasionally laden with golden-brown pigment (suspected hemosiderin).

Contributor's Morphologic Diagnosis: Liver:
a. Marked, portal-to-portal bridging fibrosis with marked ductular reaction, portal vein hypoperfusion, mild arteriolar hyperplasia, and mild lymphoplasmacytic chronic hepatitis (consistent with congenital hepatic fibrosis)
b. Moderate, multifocal to coalescing, hepatic lipidosis

Contributor's Comment: The clinical history, gross findings, and histologic findings in this case, are all consistent with congenital hepatic fibrosis (CHF). CHF is a condition resulting from abnormal development at the ductal plate affecting small interlobular bile ducts.[3,10] CHF is histologically characterized by periportal to bridging fibrosis with numerous small often irregular bile ducts and a reduction in the number of portal vein branches.[3] In humans, the fibrosis has been shown to be progressive.[10] Inflammation and cholestasis are generally absent, and regenerative hyperplasia is not typically a feature.[3] Sequelae include portal hypertension, ascites, and acquired extrahepatic portosystemic shunts. These signs may be the result of progressive fibrosis or altered and insufficient portal vein architecture.[3]

CHF is a morphologic diagnosis but not a single clinical entity and instead is the result of a spectrum of conditions that affect the ductal plate.[4] In humans, isolated CHF is rare but reported, and CHF is more often associated with fibrocystic diseases (FCDs),

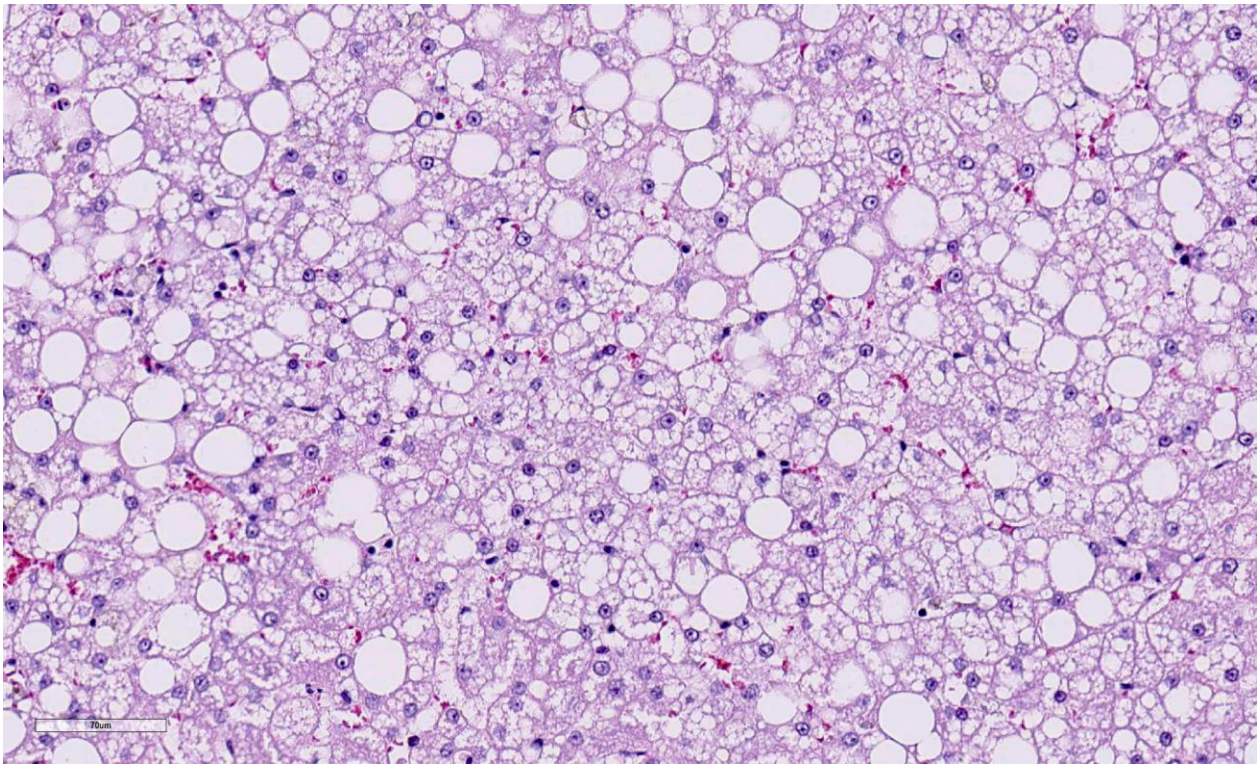


Liver, cat. Remaining hepatic parenchyma is vaguely nodular, with marked hepatocellular lipidosis and swelling. (HE, 400X)

including polycystic kidney disease (PKD).[10] While inheritance is most commonly autosomal recessive, X-linked and autosomal dominant inheritance are also described. Notably, although FCDs are currently categorized by phenotype, in the future, gene-based classification may significantly alter distinction among the FCDs.[10]

In the veterinary literature, there are relatively few reports of CHF. Species in which CHF has been reported include dogs[3,14,17], cats[1,19], aborted and neonatal calves[2,18], foals including Swiss Freiberger horses and multiple other breeds[5,11,12], an African green monkey[17], and a colony of Sprague-Dawley rats now used as a model for human autosomal recessive CHF[15]. In a case series of five dogs, all dogs presented at one year of age or younger with signs of liver disease.[3] Several other series of hepatic/hepatoportal fibrosis in young dogs have been published but do not expressly diagnose CHF versus other causes of hepatic fibrosis.[14,16]

There are two reports describing CHF in cats. One report describes CHF in cats with polycystic kidney disease (PKD), an autosomal dominant disorder for which Persian cats are predisposed.[1] Among cases of PKD, 28% had CHF, and 17% had both CHF and liver



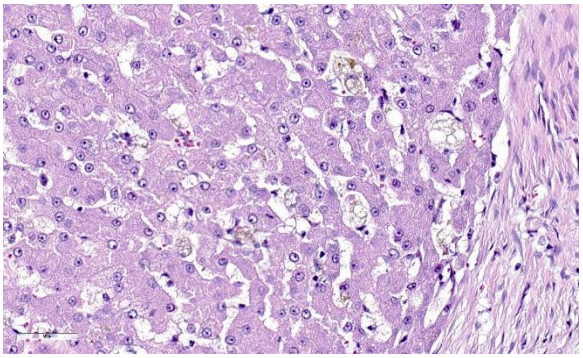
Liver, cat. Higher magnification of hepatocytes with marked swelling, lipidosis, and loss of sinusoidal architecture. (HE, 200X)

cysts. The age range for CHF-affected cats in this report was 1 to 13 years, and one of those cats had clinical signs of liver disease. [3] Additionally, there is a report of two cats with CHF and secondary acquired portosystemic shunts, and in this report, both cats also had evidence for concurrent PKO, as well as in one cat a concurrent congenital portosystemic shunt.[19] Interestingly in the case presented here, the cat lacked gross and histologic evidence of PKD, suggesting that in contrast to what is reported in the literature, CHF is not exclusively a manifestation of PKD in cats. Additionally, this case demonstrates that in contrast to dogs, CHF may progress more slowly in cats than in dogs and not become clinically relevant until later in life.

Contributing Institution:
North Carolina State University College of Veterinary Medicine
https://cvm.ncsu.edu/research/departments/dphp/programs/pathology/

JPC Diagnosis: Liver: Fibrosis, portal and bridging, diffuse, severe, with marked biliary hyperplasia, hepatocellular loss, nodular hepatocellular regeneration, and hepatocellular lipidosis.

JPC Comment: In humans, congenital hepatic fibrosis (CHF) is an autosomal recessive disease resulting from a mutation on PKDH1, whose gene product encodes fibrocystin/polyductin, a ciliary protein expressed in cholangiocytes as well as renal tubular epithelium.[6] In humans, CHF is part of the fibropolycystic diseases (FCDs), which also include autosomal dominant and autosomal recessive polycystic kidney disease, Caroli's disease, and von Meyenburg complexes (biliary hamartomas).[6,9] While a rare condition in humans (estimated in 1 in



Liver, cat. Individualized or small aggregates of macrophages contain brown lipofuscin pigment (lipogranulomas), indicative of hepatocellular loss. (HE, 400X)

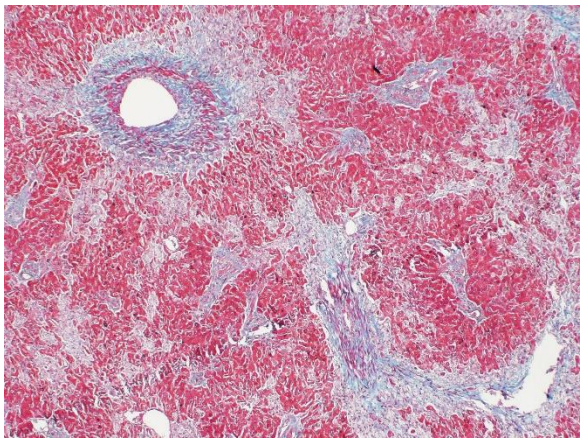
20,000 live births) it often occurs with diseases of other organs as well as other congenital diseases, including Joubert Syndrome and Bardet-Biedl Syndrome.[6]

In most other conditions resulting in progressive hepatic fibrosis. Fibrosis is a reparative response to a previous necrotizing or inflammatory insult.[6,9] In CHF, fibrosis is genetic, resulting from lack of remodeling of the ductal plate, the embryologic precursor to the intrahepatic bile ducts. The lack of remodeling ultimately results in the persistence of immature biliary structures, cystic malformation, and prominent peribiliary fibrotic responses. The actual function of polycystin is not known is thought to be involved as a regulator of transcription of various proteins involved in proliferation, differentiation, tubulogenesis and cell-matrix interactions.[6,9]

The clinical spectrum of CHF in humans is extremely broad and depends largely on whether polycystic disease is present in other organs. Cases of "pure" CHF may remain undetected into middle age. One of the most important consequences of fibropolycystic disease in humans is the development of portal hypertension, which often results in clinical symptoms of hematemesis and melena in 30-70% of cases; clinical signs related to cholangitis are less common, which may ultimately require hepatic resection or transplantation. Portal hypertension in such cases ultimately may require hepatic resection or transplant.[6,9]

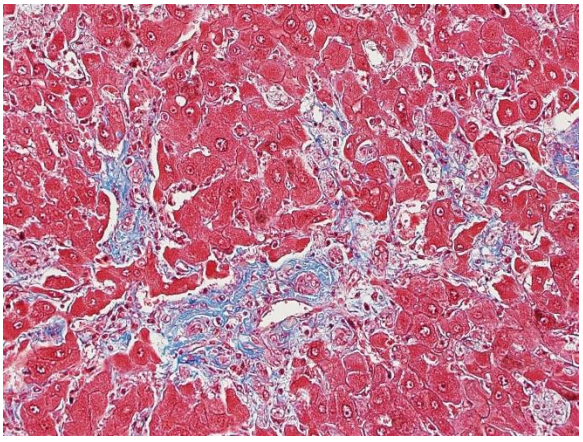
The contributor discusses a previous report of cats with CHF and PKD. Hepatic fibrosis has been reported in between 22-48% of cats with feline polycystic kidney disease, although clinical signs associated with liver failure are rare.[8] Genetic testing for PKD became available following that report, and was found to be associated with a C>A mutation in exon 29 of *PKD1* (polycystic kidney disease 1). In a feline case of combined CHF and PKD1 reported in 2015, genetic testing revealed a wild-type sequence at this position, suggesting an alternate pathogenesis, or perhaps a mutation not yet described in the cat, and is similar to the case described herein which PKD was not a concurrent condition.[8]

Upon close inspection of the submitted sections, the moderator commented on the zone of exclusion of relatively unaffected tissue. This is supplied by a subset of bile ducts of the smallest caliber at the periphery



Liver, cat. A Masson's trichrome stain demonstrates the severity of the fibrosis. There is extensive fibrosis around sublobular veins, effacing the draining lymphatics, and fibrosis bridges smaller portal triads. (Masson's, 100X)

of the liver lobe, suggesting that the process is affecting ducts of a particular size, such as the lobular and interlobular ducts rather than the smallest ductules which supply the sublobular case. The lack of pigmented macrophages suggests a limited amount of continuing damage to bile ducts at this pint in the development of this lesion. The moderator interpreted the nodularity of the parenchyma as simple entrapment of hepatocytes without the formation of regenerative nodules (which are not commonly seen in cats.)



Liver, cat. Higher magnification of the portal triads show their expansion by abundant collagen. (Masson's, 200X)

The moderator commented that hepatic fibrosis is an uncommon finding in cats except for this particular condition. In dogs, the diseases generally results in death within 6-10 months, but cats may go on for years. Dogs develop considerable portal hypertension which does not appear to be of great clinical importance in affected cats (which also much less commonly result in the development of acquired portosystemic shunts than in dogs).

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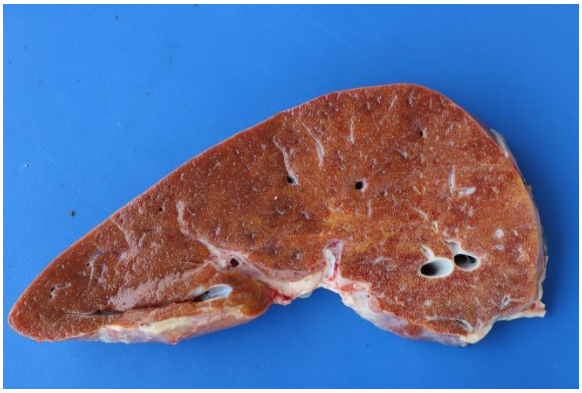
CASE II: N 544/17 (JPC 4120038).

Signalment: 24-month-old, male, Nelore (*Bos taurus indicus*), bovine

History: A lot containing 200 steers was allocated on a 125-hectare pasture (Property A) consisting of *Brachiaria brizantha*. The pasture was heavily invaded by *Crotalaria spectabilis*. The steers remained in this paddock for two months, when two of them died after showing weight loss, jaundice and increased abdominal volume. After the death of these two steers, another 150 steers of the same lot were shipped to a feedlot (Property B). After remaining for 50 days in property B, another eight steers became ill and died. Three of them were necropsied. Another 15 bovines died on property B in the subsequent two months.



Liver, ox. The liver was firm and with multifocal to coalescingt yellowish areas. (Photo courtesy of: Universidade Federal de Mato Grosso, Hospital Veterinário-HOVET, Laboratório de Patologia Veterinária, Av. Fernando Correa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT, CEP 78060-900. http://www1.ufmt.br/ufmt/unidade/?l=ppgvet)

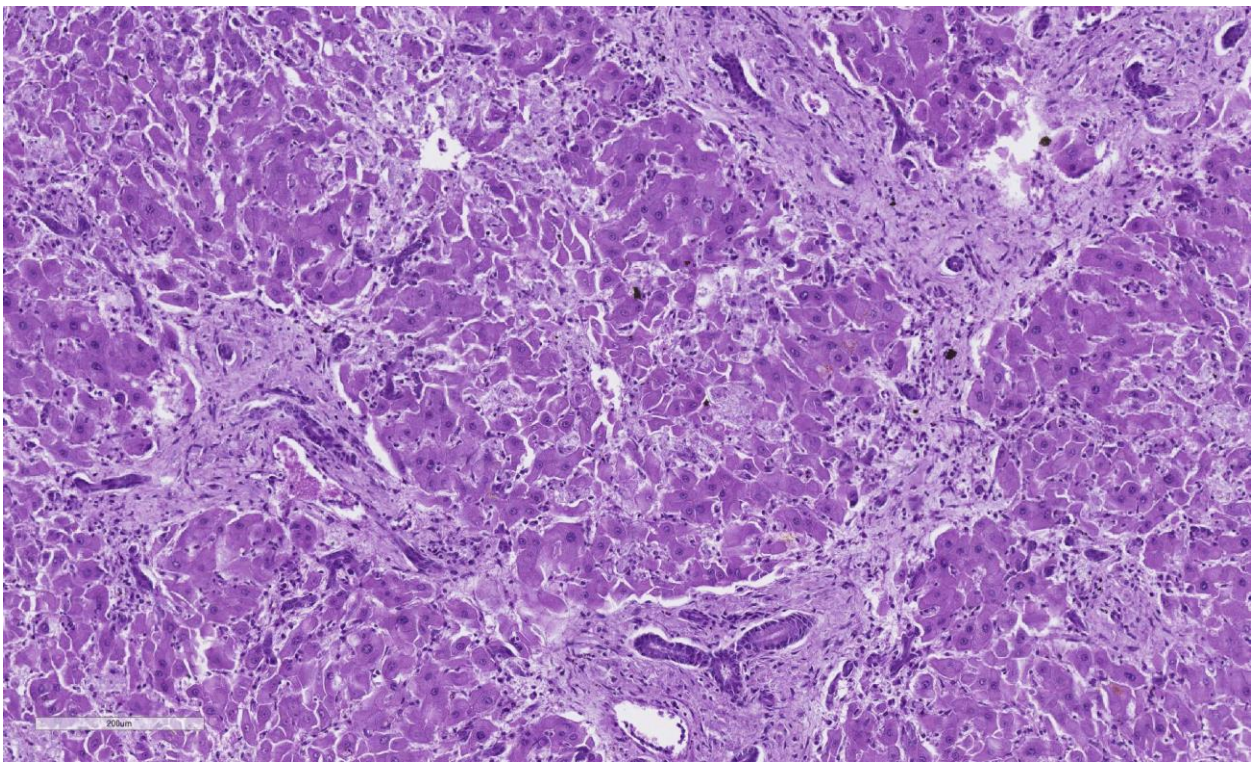
Gross Pathology: The cadaver was in poor body condition, there was about 20 liters of yellowish translucent fluid in the abdominal cavity. The liver was firm and with multifocal to coalescent yellowish areas.

Laboratory results: Analyses for aflatoxin done in the ration fed to the affected steers in the feedlot resulted negative for aflatoxin.

Microscopic Description: The liver architecture is disorganized by marked fibrosis, multifocal biliary duct hyperplasia and mild to moderate scattered hepatomegalocytosis.

Contributor's Morphologic Diagnosis: Liver, hepatocyte loss, fibrosis, bile duct hyperplasia and hepatomegalocytosis. Lesions compatible with pyrrolizidine alkaloid poisoning.

Contributor's Comment: *Crotalaria* spp. (Papilionaceae) are herbaceous plants and woody shrubs known colloquially as rattlepod or rattlebox because, as the seeds become loose within the pod, they rattle when the pods are shaken.[10] There are more than of 600 species in the genus *Crotalaria* distributed worldwide; most of these species are poisonous for livestock.[4] Forty of them were identified in Brazil and *C. mucronata*, *C. juncea*, *C. spectabilis*, and *C. retusa* were proven toxic for livestock under natural conditions. *C. mucronata* and *C. juncea* are associated with interstitial pneumonia[3], while *C. retusa* and *C. specatabilis* are associated with chronic hepatotoxicosis.[2,10] The toxic principle in *Crotalaria* spp. are dehydropyrrolizzidine alkaloids (DHPAs). DHPAs and their N-oxides are present in plant families such as Boraginaceae, Asteraceae, Orchidaceae, and Fabaceae.[8]



Liver ox. The submitted section of liver demonstrates a complex pattern of bridging fibrosis and hepatocellular loss. (HE, 130X)

DHPAs are stable chemical compounds which are biotransformed in the liver by cytochrome P-450 enzymes into toxic metabolites and pyrrole alcohols. These metabolites are alkylating agents that inhibit mitosis, resulting, in the case of the liver, in very large hepatocytes (megalocytes) – a condition referred to as hepatomegalocytosis. As the hepatocytes are lost and dropped out due to necrosis, bile duct proliferation and fibrosis occur.[6] At pasture, the Crotalaria poisoning is usually a chronic condition characterized by hepatomegalocytosis, fibrosis and bile duct proliferation.[6] However the ingestion of high doses of the plant can cause acute centrilobular necrosis.[1]

The diagnosis of intoxication by *C. spectabilis* in the steers of this report was based on clinical signs, an abundance of the plant in Property 1, and pathological changes which are similar to the ones describe in livestock poisoned by species of *Crotalaria.*[6,7] Poor body condition and ascites found in the necropsied steers ruselted from chronic hepatic disease and hepatic failure. Differential diagnosis should include other DHPAs-containing plants such as species of *Senecio*, *Cynoglossum*, *Amsinckia*, *Heliothropium*, and *Echium*. Table 1 includes some *Crotalaria* species and respective DHPAs.[4,5]

Table 1- Some *Crotalaria* species and their isolated pyrrolizidine alkaloids[4,5]

Species	Alkaloids
C. spectabilis	Monocrotaline, spectabiline, retusine
C. retusa	Monocrotaline, retusamine
C. pallida	Integerrimine, nilgirine, acetyl nilgirine, usaramine
C. incana	Anacrotine, integerrimine, usaramine
C. sagittalis	Monocrotaline

Contributing Institution:
Universidade Federal de Mato Grosso, Hospital Veterinário-HOVET, Laboratório de Patologia Veterinária, Av. Fernando Correa da Costa 2367, Bairro Boa Esperança, Cuiabá, MT, CEP 78060-900. http://www1.ufmt.br/ufmt/unidade/?l=ppgvet

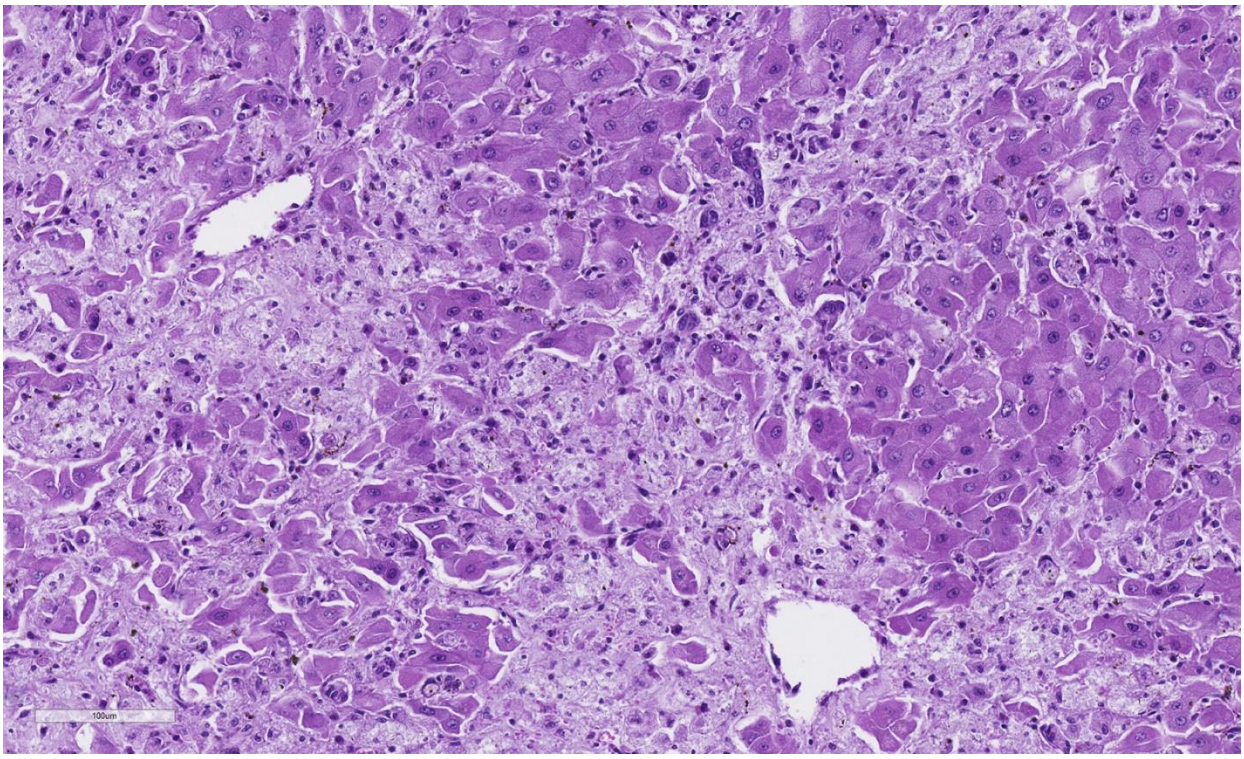
JPC Diagnosis: Liver: Fibrosis**,** portal and centrilobular, bridging, diffuse, severe, with marked hepatocellular degeneration, necrosis, and loss, lipogranuloma formation, and megalocytosis.

JPC Comment: Dehydropyrrolizine alkaloid (DHPA) toxicosis is a common toxicosis of ruminants and herbivores, with up to 350 DHPA containing plant species in the southern US alone (Paul Stromberg, personal communication).

DHPAs can produce disease in animals by a variety of acute and chronic toxicity and are documented carcinogens. The base chemical is composed of fused five-members carbons rings; toxic DHPAs have a 1.2 unsaturation. Progressive esterification of the based chemical, such as diesters and macrocyclic diesters appears to increase toxicity.[9]

DHPAs required oxidation by cytochrome P450s within the liver to develop their toxic pyrroles, which react with many essential cellular proteins (including glutathione) as well as nucleic acids. Hepatocytes, the site of activation, and endothelial cells are most often affected, resulting in hepatocellular degeneration and necrosis, with sequelae of fibrosis, biliary proliferation, and ultimately cirrhosis.[9]

Most livestock toxicity results when animals



Liver, ox: Fibrous bands extend to central veins and there is significant loss of centrilobular and midzonal necrosis. (HE, 200X)

consume toxic plants in the absence of alternative forage, but poisoning also occurs when DHPA-containing plants are harvested and fed in hay. (This type of toxicity also occurs in humans; thousands of people in Afghanistan and Tajikistan were poisoned when DHPA containing plants were harvested and processed into flour.) There is significant species variation with regard to susceptibility of DHPA toxicity, with pigs and chickens being considered highly susceptible, cattle horses, and rats moderately susceptible, and mice, sheep and goats being relatively resistant, leading to the somewhat risky "practice" of using small ruminants to clear forage areas containing plants high in PAs.

The disease in poisoned humans is somewhat different than in livestock, with endothelial damage in the liver predominating. Endothelial damage in humans leads to fibrosis of hepatic sinusoid and central veins, resulting in portal hypertension and veno-occlusive disease. Tragically, due to the susceptibility of human fetuses and neonates, they may develop fatal hepatic disease by transplacental or transmammary passage of toxic metabolites, while their pregnant or nursing mothers are unharmed.

A number of tests have been developed in recent years that help to quantitate the toxic and carcinogenic properties of various pyrollizzidine alkaloids, including an *in vitro* chicken, mouse and rat hepatocyte protocol, an *in vivo* chick system in which orally dosed chicks are given measured disease of various DHPAs and the damage to their livers measured at 7 days post inoculation, and a carcinogenic model using heterozygous p53 knockout mice which are fed DHPAs in pelleted feed for 12 months, after which they are autopsied and carcinogenic effects in various organs are compared.

The moderator marveled at the amount of macrophages present in this section, which is not especially characteristic of most cases of DHPA toxicity, but surmised that it may be another toxic principle in the pastures grazed by the affected beeves. He suggested the possibility of concurrent toxicity with a sapinogen intoxicant.

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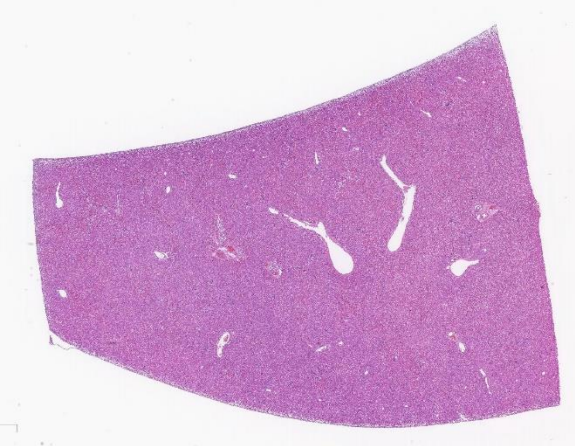
CASE III: 15-0318 (JPC 4101080).

Signalment: 6-month-old intact male Mi-Ki dog (*Canis familiars*).

History: The puppy presented with a history of lethargy, hyporexia, occasional vomiting, and abnormal behavior including opisthotonus and head pressing. A single seizure was observed, which resolved with diazepam. Following diazepam administration, the puppy became stuporous with a lack of menace response and bilaterally decreased pupillary light reflexes. The puppy's mentation gradually improved with treatment (lactulose, metronidazole, omeprazole, ondansetron and intravenous fluids with dextrose). The patient was sedated with butorphanol and alfaxalone for an abdominal ultrasound, and became obtunded



Liver, dog. One section of liver is submitted for examination. There are no apparent lesions at subgross examination. (HE, 5X)

with abnormal vocalization that persisted despite supportive care. Due to a poor prognosis, the puppy was euthanized.

Gross Pathology: The puppy weighed 1.9 kg with adequate visceral adipose. The liver was diffusely small with normal lobation, color, and consistency. A single dilated and tortuous anomalous vessel, approximately 3 mm wide, connected the gastroduodenal vein with the caudal vena cava. There was mild, bilaterally symmetric dilation of the lateral ventricles. The brain was otherwise grossly unremarkable. The lungs were mildly, diffusely wet and oozed a small amount of serosanguineous fluid and foam on cut section. Sections of lung from all lobes floated in formalin. No other gross lesions were observed.

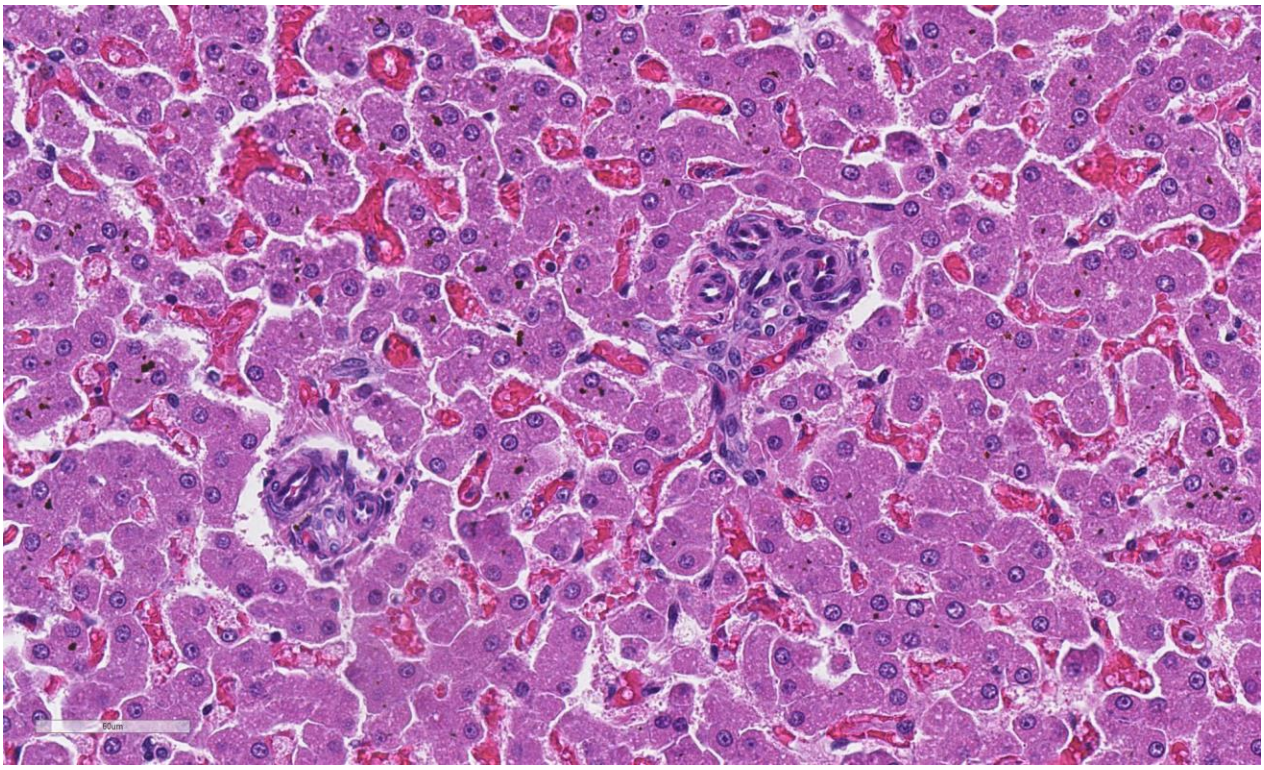
Laboratory results: Chemistry abnormalities: Albumin 2.8 g/dL, Globulins 2.2 g/dL, Creatinine 0.3 mg/dL, ALP 177 U/L, Phosphorous 6.6 mg/dL, Glucose 66 mg/dL, Cholesterol 67 mg/dL
Ammonia: 213 µmol/L (reference range: 11-35 µmol/L)
Bile acids: 112 µmol/L (pre-prandial) and 104 µmol/L (post-prandial)
Urine specific gravity: 1.026
Urine sediment: 11-20 ammonium biurate crystals /hpf

Imaging: Abdominal ultrasonography identified a small liver with a suspected single extrahepatic portosystemic shunt from the right gastric or gastroduodenal vein to the caudal vena cava.

Microscopic Description: The liver is composed of small hepatic lobules with irregularly and closely spaced portal triads. Portal triads contain increased numbers of arteriolar profiles and portal venules are often small or absent. Larger portal tracts contain dilated lymphatic vessels. Cords of hepatocytes surrounding portal tracts are thin, and individual hepatocytes are small, with a small amount of eosinophilic, occasionally finely vacuolated cytoplasm. The periportal sinusoids are infrequently dilated. Some sections contain randomly distributed small foci of inflammation composed of low numbers of lymphocytes, macrophages, and neutrophils, with individually necrotic hepatocytes. Scattered rarely throughout the parenchyma of some sections are small clusters of lipid and golden-brown pigment laden macrophages with fewer lymphocytes (pigment lipogranulomas).

In sections of brain (not submitted), the white matter, and to a lesser extent, the grey matter throughout the cerebrum and midbrain contain numerous variably sized, discrete to coalescing clear vacuoles. The lateral ventricles are mildly bilaterally and symmetrically enlarged.

Contributor's Morphologic Diagnosis: Dog, liver: hepatopathy characterized by lobular hypoplasia with hepatocellular atrophy, increased arteriolar profiles, and portal vein hypoplasia

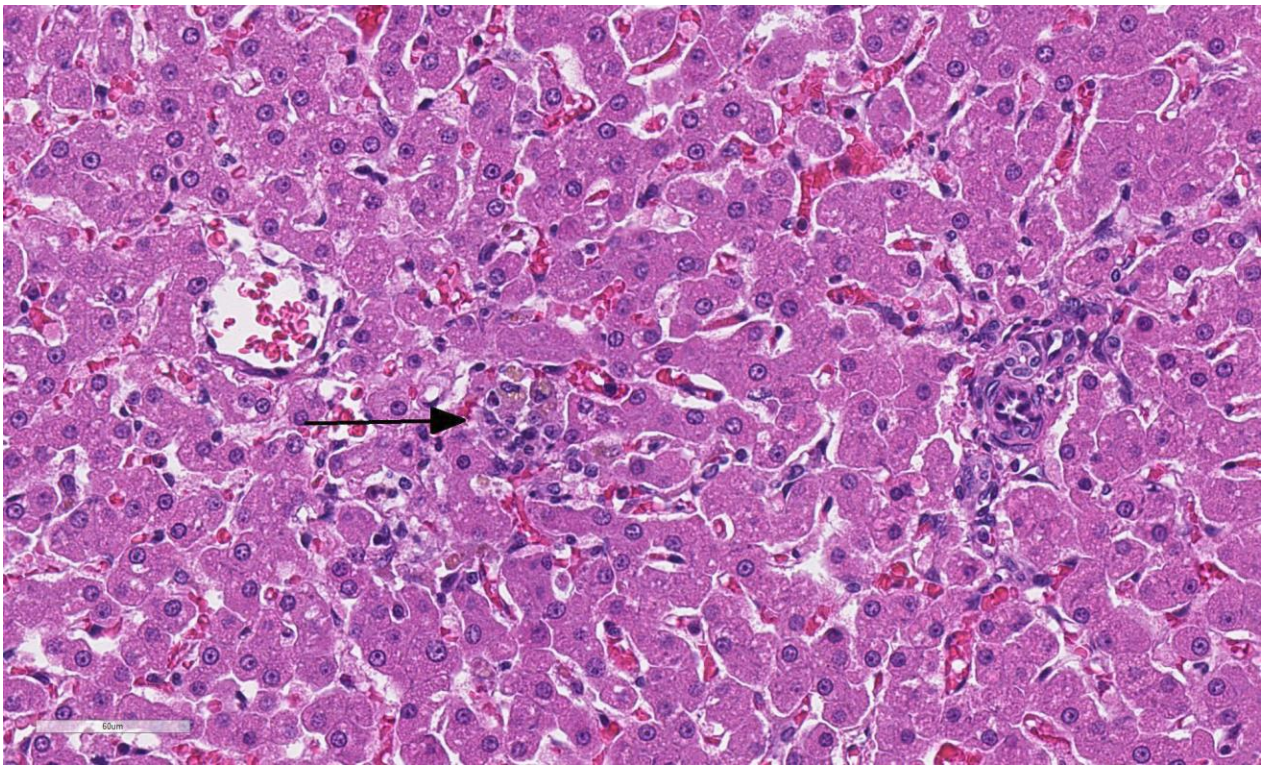


Liver, dog. Portal areas contain numerous profiles of arterioles, bile ductules, but lack profiles of portal veins. Due to hepatocellular atrophy (best adjudged by comparing the size or Kupffer cell nuclei to hepatocyte size), portal triads are often closely apposed. (HE, 400X)

Dog, liver: mild multifocal random lymphohistiocytic and neutrophilic hepatitis with single cell necrosis

Contributor's Comment: Portosystemic shunts are any anomalous connections of the portal circulation to the systemic circulation that bypass the liver. Acquired hepatic shunts are distinguished from congenital shunts on ultrasonography, exploratory laparotomy, or gross examination, as acquired shunts are typically composed of multiple anastomosing vessels rather than a single large vessel. Acquired shunts most often arise secondary to portal hypertension, such as in cirrhosis or portal vein obstruction, but can also develop in the presence of microvascular malformations[1,2].

Congenital hepatic shunts are categorized into intra- and extrahepatic shunts, and are thought to be an inherited condition in some breeds (Cairn terriers, Yorkshire terriers, Irish wolfhounds, Maltese, Australian cattle dogs)[3,4,5,6]. Large breed dogs tend to develop intrahepatic shunts, most commonly due to a patent ductus venosus[6]. Extrahepatic shunts are more common in small breed dogs and cats, most frequently connecting the portal vein, gastric vein, or splenic vein with the caudal vena cava or azygous vein[1]. Patients often present at less than 1 year of age, but can progress into adulthood before clinical signs become apparent. Clinical signs may include an overall small size compared to littermates, depressed mentation, circling, seizures, and other manifestations of hepatic encephalopathy. Clinical pathology findings can include microcytosis, hypoalbuminemia, hypocholesterolemia, hypoglycemia, low BUN, increased bile acid concentration, hyperammonemia, and ammonium biurate crystalluria[1,7].



Liver, dog. Scattered throughout the section are small aggregates of hemosiderin and lipid-laden macrophages (microgranulomas) (arrows). (HE, 400X)

Blood in the portal circulation contains hepatotrophic growth factors such as insulin, glucagon, and hepatocyte growth factor, which are essential for normal hepatocellular development[8]. Lack of blood flow and delivery of growth factors to the developing liver results in small hepatic lobules and hepatocytes, increased arteriolar profiles, small to absent portal veins, and pigment lipogranulomas[2,9]. These histologic features are characteristic of portal vein hypoperfusion; however, are not specific for a portosystemic shunt. Similar histologic lesions are also seen in cases of primary portal vein hypoplasia[1,2].

Primary portal vein hypoplasia is the current preferred term for conditions previously described as microvascular dysplasia, hepatoportal fibrosis, and idiopathic noncirrhotic portal hypertension[2]. In contrast to a portosystemic shunt, portal vein hypoplasia can result in portal hypertension, ascites, and acquired shunts[1,2]. Microscopically, portal fibrosis and bile duct hyperplasia are sometimes noted in addition to the characteristic histologic features of portal vein hypoperfusion[2,7]. Without an adequate history, clinical findings or imaging results, it is often impossible to differentiate between primary portal vein hypoplasia and congenital portosystemic shunts with histology alone. Concurrent primary portal vein hypoplasia and congenital portosystemic shunts have been reported in dogs[7].

In this case, the microscopic hepatic and brain lesions correlated well with the gross identification of a distinct anomalous vessel, clinical signs, and clinical pathologic abnormalities. This constellation of findings is consistent with an extrahepatic congenital portosystemic shunt and hepatic

encephalopathy. The cause of the mild, multifocal random hepatitis is unclear, though the distribution suggests possible early sepsis rather than an ischemic or toxic etiology.

Contributing Institution: University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology
http://www.vet.upenn.edu/research/academic-departments/

JPC Diagnosis: Liver, portal veins: Hypoplasia, diffuse, severe, with lobular and hepatocellular atrophy

JPC Comment: The contributor has provided a concise but illustrating explanation of congenital hepatic shunts and portal vein hypoplasia in the dog.

In a recent study of 125 dogs with developmental hepatic vascular disease[8], the initial diagnosis was confirmed in 89.3% of cases in which suspicion of portosystemic shunt was raised following liver biopsy, demonstrating the sensitivity of liver biopsy in such cases. In this study, animals were divided into those with intrahepatic shunts, those with extrahepatic shunts, and those with hepatic microvascular dysplasia – portal vein hypoplasia. Cases of microvascular dysplasia without apparent shunting were most commonly seen in Yorkshire terriers. In addition to more classic histologic findings of compressed lobules, multiple sections of tortuous arterioles in portal triads, and absence of portal veins within triads, this study also noted consistent but less common histologic lesions. Approximately 20% of cases of either intra- or extrahepatic shunting had hypertrophy of smooth muscle around sublobular veins, which increased to 80% in cases of microvascular dysplasia. Fibrosis of the central veins was noted in 22% of extrahepatic shunts and microvascular dysplasia but 60% of intrahepatic shunts. Calcification was visible in approximately 2% of animals with developmental hepatic vascular disease, but did not appear to be the result of dystrophic calcification of the vessel wall itself. Finally, lipogranuloma formation appeared to be most common in extrahepatic shunts (50%) but twenty percent or less in the other two categories, and tended to be more prominent in older dogs in the study.[8]

Regarding evalution of this particular specimen, the moderator made several observations. The moderator mentioned that in liver biopsies (not autopsies) you should not see sinusoids, else you are likely viewing hepatocellular atrophy. You may see it in autopsy specimens following postmortem consumption of glycogen, resulting shrinkage up to 33% of overall size. Addtionally, in humans, 20% of subcapsular portal triads lack portal veins, which could be problematic in small biopsies with few portal triads to evaluate. The moderator reiterated a generalization from his previous visits that the visualization of any lymphatics in the liver is evidence of disease but not specific to any particular one – evaluation of serum proteins, vascular or biliary disease, inflammation, etc. should all be considered, but it should never be considered a normal finding. In cases of microvascular dysplasia, dilated lymphatics is likely the result of decreased venous but normal arterial perfusion, as well as concomitant hypoproteinemia.

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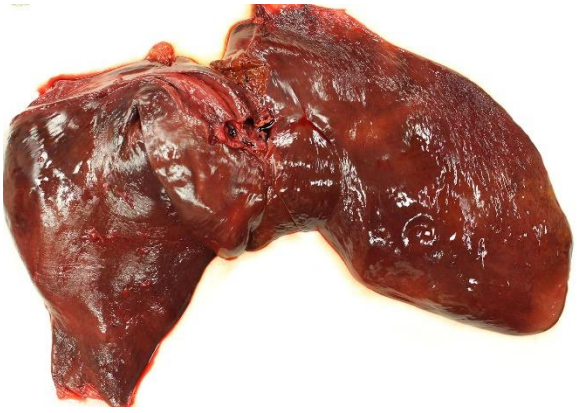
CASE IV: 19-117 (JPC 4134512).

Signalment: 15-year-old, castrated male, Quarter Horse (*Equus caballus*)

History: This horse presented to ambulatory service with an acute onset of illness. Clinical examination revealed a normal respiratory rate, normal heart rate and a temperature of 100.7 ^{o}F. There was severe icterus and dark brown urine dripped from the penis. Gut sounds were decreased in all 4 quadrants. When moved, the horse collapsed and euthanasia was elected. The horse was not vaccinated. There was no history of feed change, medications, travel or exposure to toxins.

Gross Pathology: Gross examination revealed a carcass in good nutritional condition with no evidence of dehydration and moderate autolysis. Dark red urine dripped from the prepuce. There was diffuse and severe icterus. A scant amount of serosanguinous fluid was noted in the peritoneum. The liver was small and flaccid weighing 3.8 kg or 0.7% body weight (normal range 1.2-1.5%). On section, an enhanced reticular pattern was noted. The

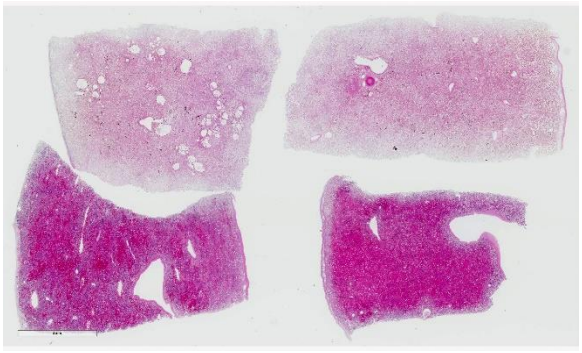


Liver, horse. The liver was small, flaccid, and weighed 3.8 kg or 0.7% body weight (normal range 1.2-1.5%). (Photo courtesy of: Diagnostic Services Unit, University of Calgary, Faculty of Veterinary Medicine, https://vet.ucalgary.ca/dsu).

small intestinal content was hemorrhagic. The urinary bladder was filled with dark red urine and the discoloration did not clear following centrifugation (pigmenturia). There was diffuse splenic congestion consistent with barbiturate euthanasia.

Laboratory results: PCR was positive for equine parvovirus (EqPV-H). PCR was negative for equine pegivirus (EPgV), non-primate hepacivirus (NPHV) or equine hepacivirus and Theiler's disease-associated virus (TDAV). PCR was negative for *Leptospira* species.
Urine sediment: 11-20 ammonium biurate crystals /hpf

Microscopic Description: Liver: Four sections are available for examination with varying degrees of autolysis and postmortem bacterial overgrowth. Diffusely, the hepatic lobules are distorted and reduced in size owing to marked loss of centrilobular and midzonal hepatocytes. This is accompanied by stromal collapse and sinusoidal congestion. Individualized periportal hepatocytes remain along the periphery of the lobules. Periportal hepatocytes have abundant cytoplasm containing multiple, large, well-defined vacuoles consistent with macrovesicular fatty change. Large, multinucleated hepatocytes are frequently observed (hepatocellular giant cell or syncytial cell formation). Kupffer cells contain a moderate amount of light brown, granular pigment. The portal units are expanded by minimal fibrosis, infrequent duplication of the bile ducts and a scant infiltrate of mononuclear inflammatory cells including lymphocytes, histiocytes and plasma cells. Copper was not observed on rhodanine stain and a scant amount of iron restricted to the Kupffer cells was noted on Perls' Prussian blue stain.

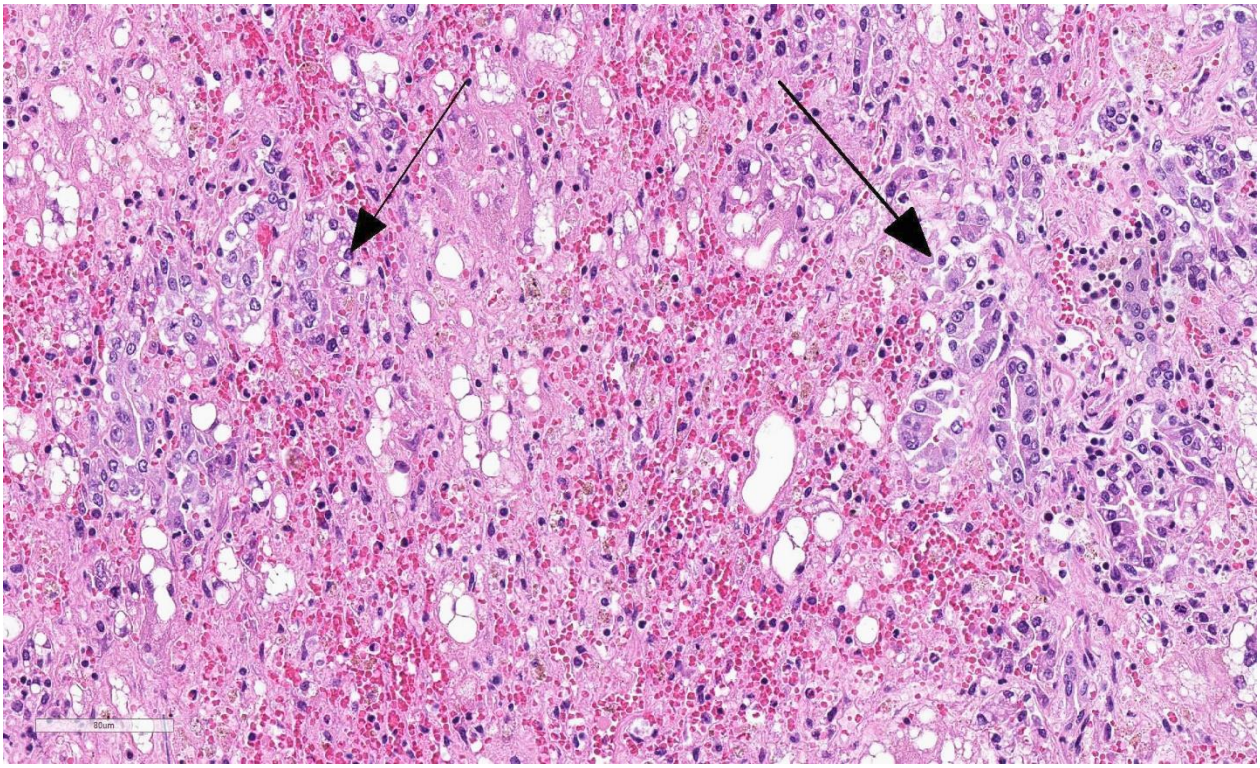


Liver, horse. Four sections of liver are submitted for evaluation; the two sections at top are poorly preserved. (HE, 5X)

Kidney (not shown on slide): Multifocally renal tubules are ectatic, are lined by attenuated epithelium and contain granular to hyaline, bright orange to red material interpreted to be heme pigment.

Contributor Morphologic Diagnosis: Liver: Hepatocellular loss and degeneration, submassive, severe, subacute with stromal collapse, mild portal hepatitis and syncytial cell (giant cell) formation

Contributor Comment: Based on the clinical course of disease, gross pathology, histopathology, PCR results and lack of exposure to known hepatotoxins, this is interpreted to be a case of equine serum hepatitis or Theiler's disease. Serum hepatitis is a long recognized cause of acute liver failure in horses. Theiler's disease was first described in 1919 in horses following vaccination using equine antiserum against African horse sickness.[4,13] The disease is reported globally and is typically observed following administration of equine biological products including tetanus antitoxin, botulinum antitoxin, antiserum against *Streptococcus equi*, pregnant mare's serum and equine plasma.[5,14] Of these products, serum hepatitis is most commonly associated with tetanus antitoxin possibly because it is the most frequently used.[14] Similar to the current case, a number of cases of equine



Liver, horse. There is almost total loss of hepatocytes between portal triads (arrows). The intervening space contains abundant hemorrhage, numerous siderophages, and few degenerating remnant hepatocytes with contain one or more large clear vacuoles within their cytoplasm. (HE, 267X)

serum hepatitis are not associated with administration of equine biologicals raising the specter of horizontal transmission from contaminated medical equipment and insect vectors such as tabanid flies. [2,3,15]

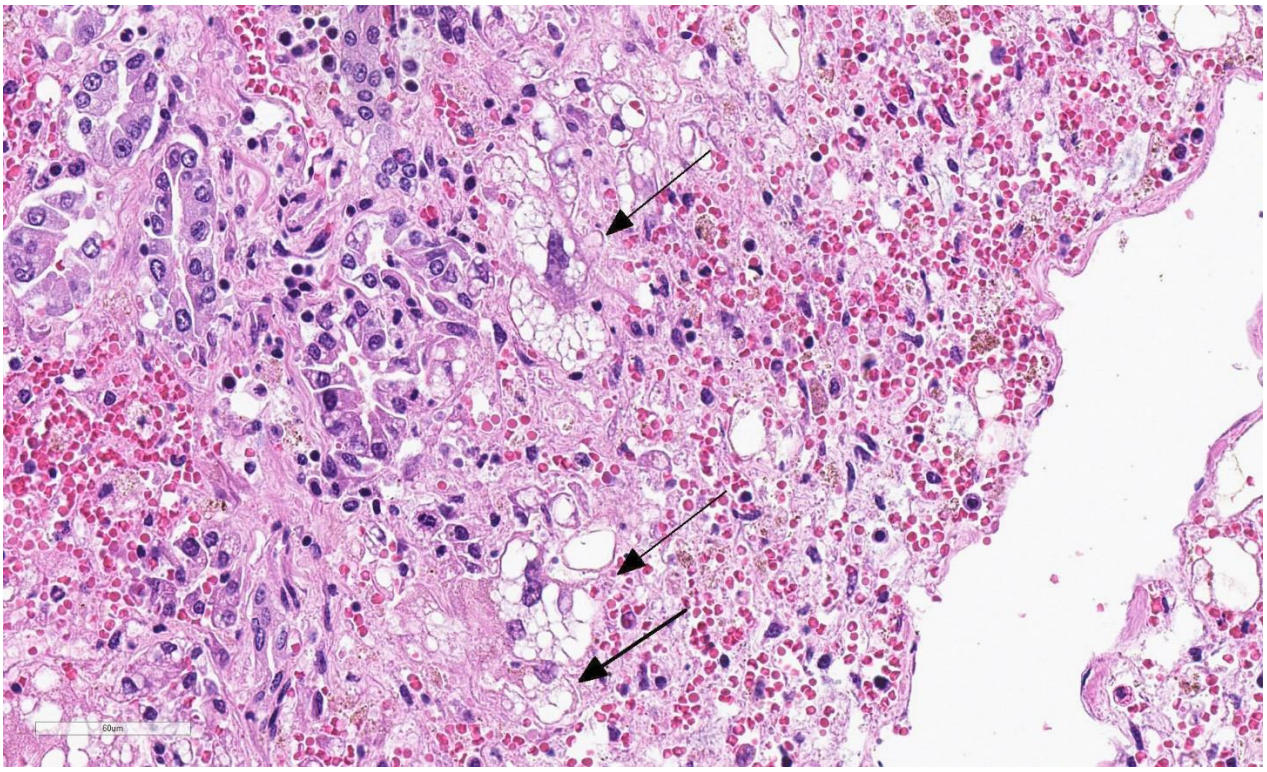
The typical incubation period is 4-10 weeks, but can be as long as 14 weeks.[4,5,14] The onset of clinical disease is sudden with rapid progression to death. Lethargy, icterus, photosensitivity, fever and neurological signs including hyperexcitability, blindness and ataxia are reported.[4,13] Morbidity rates vary from 1-18% in outbreaks and mortality rates are between 50-90%.[14]

Typical biochemical findings include marked elevation in liver enzyme values (primarily SHD and AST and to a lesser degree GGT), direct and indirect bilirubin and bile acids.[5,14] Reported gross lesions include icterus, ascites, serosal and renal petechiae, and intestinal hemorrhage. The liver is small and limp due to the hepatocellular loss and is often described as having a "dishrag" appearance. Cut surface reveals a highlighted zonal pattern.[2, 4] Acute hepatic necrosis and hemorrhage are not features of serum hepatitis. Microscopic features are instead more chronic than the clinical course suggests and are dominated by hepatocellular loss with collapse and distortion of the reticulin framework as seen in the current case. There may be few surviving periportal hepatocytes which demonstrate hepatocellular vacuolation consistent with macrovesicular lipidosis. Additional microscopic features include variable numbers of apoptotic hepatocytes, deposition of bile pigments in Kupffer cells and hepatocytes, mild fibroplasia in the portal units and variable ductular proliferation. Inflammation is not robust, but a diffuse infiltrate of lymphocytes, plasma cells,

histiocytes and neutrophils may be present.[2, 4]

While the association between serum hepatitis and administration of equine biologics has been known for 100 years and a blood-borne virus has long been suspected, the etiologic agent of Theiler's disease has eluded the veterinary profession. Since 2012, four emerging viruses have been identified and studied for their possible association with equine hepatitis. Three are in the family *Flaviviridae* including equine pegivirus (EPgV), non-primate hepacivirus (NPHV) or equine hepacivirus, and Theiler's disease-associated virus (TDAV). The fourth is equine parvovirus-hepatitis (EqPV-H). NPHV, closely related to hepatitis C virus, is hepatotropic and hepatic disease following experimental transmission is documented.[9] TDAV was first identified in an outbreak of acute clinical hepatitis in horses following administration of botulinum antitoxin of equine origin.[3] However, retrospective analysis of samples indicated the presence of EqPV-H in all TDAV positive samples indicating coinfection initially missed do to methodology.[5] EPgV is a common infection of horses in the United States and Western Europe, but the virus is not hepatotropic and has not been associated with hepatic disease.[6] Recently, EPgV infection was associated with a reduced risk of having increased liver enzyme activity indicating that EPgV is an unlikely cause of equine hepatitis.[10] In 2018, a novel equine parvovirus (equine parvovirus-hepatitis, EqPV-H) was discovered in the liver and serum of a horse succumbing to Theiler's disease and experimental administration of EqPV-H positive tetanus antitoxin samples resulted in viremia and subclinical or clinical hepatic disease in 2 horses. This study also revealed EqPV-H viremia in 13% of normal horses tested suggesting that many infected horses don't develop clinical disease.[5] More recently, 2 companion studies demonstrated EqPV-H infection in 18 consecutive cases of serum hepatitis and EqPV-H infection in 9/10 horses with Theiler's disease in the absence of equine biologic product administration. In both studies, the 3 additional flaviviruses implicated in equine hepatitis were absent or rarely present.[14,15] These recent studies support the role of EqPV-H in Theiler's disease, but further studies are required to unequivocally demonstrate that EqPV-H is the cause of serum hepatitis.

In the standard veterinary pathology textbooks, giant cell formation is not described as a feature in equine serum hepatitis and was an unexpected feature in this case. There is a single report of Theiler's disease in a Canadian horse in which multinucleated hepatocytes are described.[13] Giant-cell hepatitis (GCH) is well-described in humans and is characterized by parenchymal inflammation with formation of large multinucleated hepatocytes.[11] It is most commonly seen in neonates, and occurs rarely in adults where it is known as post-infantile GCH.[11,16] It is considered to be a nonspecific tissue reaction to various stimuli and therefore is not specific to an etiology. The pathogenesis is unknown and may be due to hepatocyte nuclear proliferation without subsequent cell division or membrane fusion of neighboring hepatocytes.[11] In infants, a recent study indicated that most cases of GCH were idiopathic. In this case series, panhypopituitarism was the most common recognizable clinical association with fewer cases attributed to biliary atresia, Alagille syndrome, bile salt defects, neonatal hemochromatosis, SCID, and viral infections.[16] In adults, giant cell transformation occurs with a variety of insults including viral hepatitis (hepatitis A, hepatitis B, non A, non B hepatitis, hepatitis C, and Epstein-Barr virus), autoimmune hepatitis, and with a variety of drugs and herbal remedies.[11] Giant cell hepatitis is an



Liver, horse. Remaining periportal hepatocytes (located adjacent to portal triads) (arrows)are expanded by numerous clear lipid vacuoles. (HE, 400X)

uncommon lesion in animals but has been recorded in cats, calves and foals.[4] In foals, giant cell transformation has been reported in cases of neonatal isoerythrolysis[1] and in aborted foals with suspected leptospirosis.[17]

In the absence of muscle injury in this horse, the dark red appearance to the urine and pigmentary nephrosis were interpreted as hemoglobinuria and hemoglobinuric nephrosis indicative of intravascular hemolysis. Common causes of intravascular hemolysis and hemoglobinuria in horses include immune-mediated hemolytic anemia; a number of infections including piroplasmosis, equine infectious anemia, leptospirosis, and clostridial hepatitis; drug toxicity; toxic plants including red maple, *Brassica* species and membranes of the onion family; and hepatic failure. Hemolysis secondary to hepatic failure is presumed in this case and intravascular hemolysis is described in the terminal stages of Theiler's disease.[2] The mechanism for intravascular hemolysis in cases of liver failure in horses is unknown; however, bile acids or their salts are considered possible hemolytic factors.[8]

Contributing Institution:
Diagnostic Services Unit
University of Calgary, Faculty of Veterinary Medicine
https://vet.ucalgary.ca/dsu

JPC Diagnosis: Liver: Degeneration, necrosis and loss, massive, diffuse, severe, with stromal collapse and hepatocellular lipidosis**.**

JPC Comment: The contributor has done an outstanding job summarizing the history, pathogenesis, and current thought regarding this very dramatic and well-known disease of the horse.

Sir Arnold Theiler 1867-1936), for which this disease still bears his name, was a Swiss veterinarian and researcher of great importance in the area of veterinary research and education in South Africa. As the state veterinarian for the South African Republic, he developed he first vaccine against rinderpest. In 1919, he was the first to describe acute serum hepatitis in animals vaccinated against African horse sickness. As the first director of the Onderstepoort veterinary Research Institute, he was instrumental in leading the research team in their investigations of many diseases of the region including East Coast fever (caused by *Theileria parva),* sleeping sickness, heartwater, malaria, and African Horse sickness. He shortly became the dean of the newly built University of Pretoria Faculty of Veterinary Science, and worked there until his passing.

Since the identification of an equine parvovirus as the putative agent of equine serum hepatitis, a number of groups have begun the process of determining the extent of its presence in a variety of equine biologics, including horse serum from a variety of providers. In one study,[7] 11 out of 18 samples of serum from providers in the US, Europe, Canada, New Zealand and South America demonstrated the presence for equine parvovirus-hepatitis (EqPV-H) using gel electrophoresis of the qPCR products and anti-EQPv-H antibodies were also detected in the same samples, although both fetal-based serum products tested negatively. While the presence of the putative agent has only been definitively identified in conjunction with disease in the US and China at this time, this study shows that the putative agent is truly of global origin.[7]

One of the classic syndromes associated with equine serum hepatitis and the reason it has longed been considered an infectious disease rather than just a reaction to biologics is the propensity for horses in contact to develop the disease. A 2018 study[15] reports the results of identification of EPv-H from 10 cases of apparent serum hepatitis on 6 different properties and the results of screening of in-contact horses which had not previously received any equine biologicals. In this study, animals with clinical infection were 80% positive, while in-contact animals were 48% positive, suggesting that these animal may be subclinical infected. Samples from infected animals were also tested for other viruses that had previously been associated with equine serum hepatitis (non-primate hepacivirus A), EPgV (pegivirus E) and EDAV (pegivirus D) were not identified in this study. [15]

Due to the massive degeneration, necrosis and hemorrhage affecting much of the four sections on the slide, this proved to be a deceptively difficult slide to describe. A common artifact was present in many of the slides which appears as blue spicular crystals within vacuoles of fat which was interpreted as calcium stearate crystals.

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