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Conference Moderator:
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CASE I: 09021035 (AFIP 3136277).

Signalment: 3.5-week-old, female, Angus calf (*Bos taurus*).

History (per referral): This calf was born on 2 February 2009 with no known complications. The patient was lethargic post partum and was administered synthetic colostrum, florfenicol (6 mL), and ceftiofur hydrochloride. The patient never improved and was transported to the referring veterinarian. The referring veterinarian felt there was a liver abnormality based upon severe icterus and treated the calf with antimicrobials and vitamin injections. The patient did not improve and was referred to Oklahoma State University. Physical exam at OSU revealed severe icterus. Ultrasound revealed hepatomegaly and hyperechoic liver parenchyma, and the gallbladder could not be located. A congenital biliary anomaly was suspected.

Gross Pathology: The patient was in good body condition. The mucous membranes and sclerae were dark yellow. Internally, the subcutaneous fat, retroperitoneal and pelvic canal fat, intra-abdominal fat, joint fluid, and cerebrospinal fluid were deep yellow. The liver was enlarged with rounded margins and the capsular surface was mottled red and yellow. The hepatic parenchyma was diffusely and markedly firm. The gallbladder was not present, and while the duodenal papilla was present, a discernible bile duct was not observed to communicate with the papilla. A 2.5 cm diameter focus of gelatinous yellow tissue was present in the region where the gallbladder was expected to be located.

Histopathologic Description: The normal lobular architecture of the liver is disrupted by tracts of proliferative, branching and anastomosing, hyperplastic bile ducts surrounded by moderate to marked amounts of fibrous connective tissue (confirmed with trichrome stain). These tracts extend between adjacent portal triads and often to the central veins. Hepatocellular cords are separated into small islands by the bands of fibrous connective tissue and hyperplastic bile ducts. Golden bile pigment (confirmed with Hall's special stain) forms multiple lakes that often replace hepatocytes and are scattered throughout the hepatic parenchyma. Bile pigment is also often present within multinucleated giant cells.

The small tissue specimen from the region of the gallbladder is composed largely of fibrous connective tissue that contains few bile duct profiles nested together peripheral to a larger, central, epithelial-lined ductal or aplastic bladder-like structure. Scattered at the borders of the tissue are small amounts of smooth muscle.

Contributor's Morphologic Diagnosis: 1. Liver: Severe intrahepatic bile duct hyperplasia and periportal fibrosis with marked hepatocellular loss and severe, multifocal cholestasis with bile lake formation.
2. Gallbladder and duct: Gallbladder aplasia with extrahepatic biliary atresia.

Contributor's Comment: Congenital biliary atresia is discontinuity or obstruction of the biliary tree (extrahepatic or intrahepatic) that results in severe icterus and liver damage in young patients. The disease is rare, though well-described in people, and the defect occurs alone or in combination with other congenital defects. The condition has been reported in several veterinary species including foals, lambs, calves, cats and dogs.(1,4-6) Whether or not gallbladder agenesis/aplasia is included or encompassed within the diagnosis of biliary atresia is variable between reports. Since it is well documented that biliary atresia can be intrahepatic or extrahepatic (or both) and occurs with or without gallbladder lesions, it is probably best that the two conditions remain separate morphological diagnoses.

In reports from people and animals, livers have been described as macroscopically large or shrunken. The histological lesion in the liver is more similar across species and characterized predominantly by portal and bridging fibrosis with either an absence of bile ducts (intrahepatic atresia) or marked proliferation of bile ducts (in extrahepatic atresia). There is marked cholestasis. The hepatic histological lesion is similar to that of calves with congenital hepatic fibrosis syndrome; therefore, evaluation/verification of the gallbladder and bile duct is important in the delineation of these syndromes.

The etiology of biliary atresia in man and animals is debatable. Most follow two pathways of either congenital defects in morphogenesis or post-formation destruction (whether prenatal or postnatal), usually blamed on infection/ inflammation.(1,2) Defects in morphogenesis are suspected to be either inherited or secondary to gestational toxin exposure.(2,5) Post-formation inflammatory destruction of the biliary tree has been blamed on viral infections and immune-mediated destruction in people and severe biliary ascarid invasion with subsequent inflammation and blockage in the dog.(2,6)

AFIP Diagnosis: Liver: Bridging fibrosis, diffuse, marked, with biliary hyperplasia, hepatocellular loss, canalicular cholestasis and bile granulomas.

Conference Comment: We are grateful to the contributor for furnishing this very instructive case. As is customary at the AFIP, conference participants were denied prior access to the submitted history, necropsy findings, and gross image; while creating a rather artificial handicap, the method provides for a rich educational opportunity, primarily by reducing bias and thus stimulating discussion. Uniformly, conference participants strongly suspected a toxic etiology in this case based upon the diffuse distribution of the lesions. More specifically, participants interpreted the prominent bridging fibrosis and loss of hepatocytes as consistent with chronic hepatointoxication. The striking lesions attributable to cholestasis (i.e. bile lakes and granulomas; ectatic, albeit usually empty, bile canaliculi; and biliary hyperplasia) led many participants to precisely implicate toxins that target the biliary epithelium, such as the mycotoxin sporidesmin and the plant *Tribulus terrestris*, which causes a toxicosis known as "geeldikkop" in sheep. Careful examination of the specimen, however, reveals compelling evidence against both of these toxins, such as the paucity of inflammation, besides that which is associated with bile lakes (bile granulomas). Sporidesmin, produced by the fungus *Pithomyces chartarum*, concentrates in the bile, directly irritating the connective tissues of the portal tracts and bile ducts, and at high concentrations, causes biliary epithelial necrosis; the result is acute cholangitis or cholangiohepatitis. Although generally mild, the inflammation in sporidesmin toxicosis is typically more severe than that present in this case. Moreover, sporidesmin causes extensive necrosis of bile duct epithelium with sloughing of necrotic debris into the lumen, features lacking in this case. Saponins of the plant *Tribulus terrestris* are likely responsible for geeldikkop ("yellow bighead") in sheep, which is distinguished grossly by the presence of white, semifluid, crystalline material in the cystic and larger intrahepatic bile ducts. Histologically, hepatocyte vacuolation and Kupffer cell hyperplasia are characteristic of the acute toxicosis, whereas crystalline material within the bile ducts and hepatocytes is more obvious in chronic intoxication; none of these is a feature of the present case. (7)

This exercise underscores the value of resisting the temptation to commit to just one category of etiology (e.g., toxic, infectious, congenital, degenerative, neoplastic, etc.) without first giving due deliberation to the other potential categories that may produce a similar constellation of microscopic lesions. The clinical history and gross findings substantially amplify the index of suspicion for a congenital abnormality in this case. That said, the cause of biliary atresia remains enigmatic, and indeed the condition may represent a common phenotypic result of a number of different causes, including inherited defects and/or in utero intoxication targeting the biliary epithelium. In support of the latter, the pyrrolizidine alkaloid-containing plant, *Senecio* sp., has been implicated as the cause of *in utero* liver damage in calves whose dams ingested the plant during pregnancy.(3) Intriguingly, a 1988 outbreak of congenital biliary atresia in lambs and calves in New South Wales, Australia, occurred at the same site as a similar outbreak in lambs in 1964. Epidemiologic evidence, including the simultaneous occurrence in both calves and lambs in the second outbreak, argued against an inherited defect, and signified that the lesion may have stemmed from a toxic or infectious insult to the developing fetuses that produced choledysgenesis and biliary atresia *in utero*; although several suspect weeds were investigated, a specific etiology was not confirmed.(5)

This case was reviewed in consultation with the AFIP Department of Hepatic Pathology, which noted remarkable histomorphological similarities with cases of biliary atresia in human neonates. Although only rarely reported in other species, biliary atresia in humans is the most common cause of neonatal cholestasis and the most common indication for pediatric liver transplantation; yet, its cause remains obscure, with evidence put forth in support of

five possible mechanisms: 1) viral infection, 2) environmental toxin exposure, 3) immunologic/inflammatory dysregulation, 4) defective biliary tract morphogenesis, and 5) defective fetal/prenatal circulation.(2)

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References:

1. Bastianello SS, Nesbit JW: The pathology of a case of biliary atresia in a foal. J S Afr Vet Assoc **58:**89-92, 1987
2. Bezerra JA: Potential etiologies of biliary atresia. Pediatr Transplantation **9:**646-651, 2005
3. Fowler ME: Pyrrolizidine alkaloid poisoning in calves. J Am Vet Med Assoc **152:**1131-1137, 1968
4. Hampson ECGM, Filippich LJ, Kelly WR, Evans K: Congenital biliary atresia in a cat: a case report. J Small Anim Pract **28:**39-48, 1987
5. Harper P, Plant JW, Unger DB: Congential biliary atresia and jaundice in lambs and calves. Aust Vet J **67:**18-22, 1990
6. Schulze C, Rothuizen J, van Sluijs FJ, Hazewinkel HA, van den Ingh TSGAM: Extrahepatic biliary atresia in a border collie. J Small Anim Pract **41:**27-30, 2000
7. Stalker MJ, Hayes MA: Liver and biliary system: toxic hepatic disease. *In:* Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, pp. 372-378. Saunders Elsevier, Philadelphia, PA, 2007

CASE II: 6860-09; 8358-08 (AFIP 3149858).

Signalment: Juvenile, female herring gull (*Larus argentatus*).

History: Several juvenile herring gulls were found dead.

Gross Pathology: All examined gulls were in very poor nutritional condition. The air sacs contained numerous greenish plaques and the lungs were full of disseminated yellow nodules typical of aspergillosis. The spleen was enlarged. The bursa of Fabricius contained few *Ichthyocotylurus platycephalus* trematodes.

Laboratory Results: *Aspergillus fumigatus* was isolated from the lungs and air sacs, and *Salmonella* Typhimurium phage type 41 from the intestines and liver. Avian influenza virus (not H5 or H7) was shown with RT-PCR from tissue samples.

Histopathologic Description: Bursa of Fabricius: There is diffuse severe lymphoid depletion in the cortex and medulla of bursal follicles. Numerous large basophilic intracytoplasmic inclusion bodies, often in clusters (botryoid), are seen both in the cortex and medulla, in the cytoplasm of macrophages or vacuolated reticular cells. Some follicles have cystic lumina with pseudostratified epithelium. Trematode eggs can be seen in some sections in the bursal lumen.

Contributor's Morphologic Diagnosis: Bursa of Fabricius: Lymphoid depletion, subacute, severe, with numerous intrahistiocytic intracytoplasmic inclusions, consistent with avian circovirus infection.

Contributor's Comment: Botryoid bursal inclusion bodies are considered pathognomonic for avian circovirus infection.(1) Circovirus-like disease has been reported in several countries in die-offs of young gulls since 1999.(1) Birds have typically been severely emaciated, and often affected with secondary aspergillosis and/or salmonellosis, as in this case.(1,2) Large intracytoplasmic basophilic/amphophilic inclusion bodies have been detected in macrophages and in lymphocytes in atrophic bursal follicles and in the spleens of these birds.(1) Circovirus-like virions have been demonstrated in these lesions with electron microscopy.(1) Recently, *in situ* hybridization with a pigeon circovirus probe was used to demonstrate circovirus in gulls from Sweden and New Zealand.(2) Avian circovirus has not been isolated in cell cultures thus far. The epidemiology of this disease in wild birds is largely unknown.

Circoviridae are small, nonenveloped viruses with a circular, single-stranded DNA genome. Infections caused by circoviruses are often subclinical or cause immunosuppression. Virions are highly stable in the environment. The family Circoviridae consists of two genera: Circovirus and Gyrovirus.(3) Chicken anemia virus, which is the best

known of the circoviruses, belongs to Gyrovirus. It causes chicken infectious anemia (CIA), and has not been detected in species other than the chicken.(3) It causes atrophy of bone marrow hemocytoblasts and T-cells in lymphoid tissue with consequent aplastic anemia and immunosuppression in 1-3 week old chicks.(3) Histologically, lymphoid and bone marrow atrophy is detected.

Porcine circovirus (PCV) 1 and 2, psittacine beak and feather disease (PBFD) virus, and avian circoviruses are members of the genus Circovirus.(3) These viruses cause a rather similar disease in different species.(3) PCV2 is the cause of post-weaning wasting syndrome in pigs. Weight loss, secondary infections and lymphadenopathy are seen mainly in approximately 6-week-old pigs. Granulomatous inflammation with severe lymphoid depletion, histiocytosis, giant cells and large basophilic botryoid intracytoplasmic inclusion bodies are seen in macrophages in several tissues.

Psittacine beak and feather disease virus infects the basal layer of the epidermis and the monocyte-macrophage system of young (under 3 years old) psittacines and causes immune suppression, beak and feather deformities and baldness.(1) The disease can be also acute or peracute in neonates or very young birds. Basophilic intracytoplasmic or intranuclear, often large, botryoid inclusion bodies are seen in feather follicle epidermis or in macrophages in the bursa of Fabricius or thymus.

Other avian circoviruses have been discovered from several species (e.g. gulls, pigeons, goose, ducks, canaries, finches, ostriches).(1,2) It seems likely that the infection is much more prevalent than the disease; virus can be found in clinically normal birds.(3) The disease seen in pigeons and geese resembles that detected in gulls, with immunosuppression, lymphoid depletion and secondary infections.

Virus replication takes place in actively dividing cells, like the basal epithelial cells in PBFD. Inclusions in macrophages are likely to be phagocytosed material.(3) Lymphoid depletion has been speculated to be induced by cytokines, not due to direct viral infection of the lymphocytes.(3)

AFIP Diagnosis: 1. Bursa of Fabricius (cloacal bursa): Lymphoid depletion, diffuse, marked, with numerous intrahistiocytic intracytoplasmic botryoid inclusion bodies, etiology consistent with circovirus.
2. Bursa of Fabricius (cloacal bursa): Intraluminal trematode eggs.

Conference Comment: The contributor provides a succinct discussion of this interesting entity, and the notes on salient comparative pathology findings are especially relevant. Accordingly, conference participants readily attributed the distinctive intrahistiocytic intracytoplasmic botryoid inclusion bodies in the submitted sections of cloacal bursa to circoviral infection, noting their conspicuous resemblance to those characteristically encountered in the lymph nodes of pigs with post-weaning multisystemic wasting syndrome (PMWS). Readers are urged to review WSC 2009-2010, Conference 3, case IV for additional details regarding circoviruses in general, and in particular, the contentious role of PCV2 in porcine dermatitis and nephropathy syndrome (PDNS). Attendees briefly reviewed the histology and function of the cloacal bursa by examining the section from a normal chicken submitted with WSC 2009-2010, Conference 15, case III. In addition to the microscopic lesions described by the contributor, some participants' slides featured small foci of fat atrophy at the periphery of the section, a finding consistent with the reported gross lesions.

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References:
1. Pare JA, Robert N: Circovirus. *In:* Infectious Diseases of Wild Birds, eds. Thomas NJ, Hunter DB, Atkinson CT, pp.195-205. Blackwell Publishing, Ames, IA, 2007
2. Smyth JA, Todd D, Scott A, Beckett A, Twentyman CM, Bröjer C, Uhlhorn H, Gavier-Widen D: Identification of circovirus infection in three species of gull. Vet Rec **159:**212-214, 2006
3. Todd D: Avian circovirus diseases: lessons for the study of PMWS. Vet Microbiol **98:**169-174, 2004

CASE III: N06-358-9 (AFIP 3036133).

Signalment: 29-year-old, 16.27 kg, female Baboon (*Papio* sp.).

History: This baboon was selected for euthanasia due to dark loose stools and senescence.

Gross Pathology: On external examination the animal was found in good body condition and adequately hydrated. Gross findings included multiple, pale tan to white, irregularly shaped, firm nodules, up to 5 cm in diameter, multifocally distributed in the hepatic parenchyma.

Histopathologic Description: On light microscopy the liver architecture was multifocally effaced and replaced by a well demarcated, unencapsulated, infiltrative, highly cellular neoplasm composed of nests, packets, and cords of epithelial cells often forming glandular structures (rosettes and pseudorosettes), supported by a fine to moderate fibrovascular stroma. The neoplastic cells were cuboidal to columnar with variably distinct cell borders and a moderate amount of eosinophilic, finely granular cytoplasm. The nuclei were central to basilar, round to oval, with coarsely stippled chromatin. Mitoses were rare (less than 1 per 10 HPF, 400X magnification). Additional features included scattered central areas of necrosis and hemorrhage, and variable amounts of homogeneous, eosinophilic material filling the central lacunae of the rosette formations. The adjacent portions of the non-affected liver parenchyma showed mild to moderate atrophy from neoplastic compression.

By immunohistochemistry the neoplastic cells were positive for pancytokeratin, chromogranin A, NSE and synaptophysin and were negative for vimentin, S100 protein, glucagon and insulin.

Contributor's Morphologic Diagnosis: Liver: Carcinoma, neuroendocrine, Baboon, *Papio* sp.

Contributor's Comment: The morphologic features, along with the positive staining for the neuroendocrine markers described above, led to the classification of this neoplasm as a neuroendocrine carcinoma.(1) No similar neoplastic changes were observed in all the other tissues examined; therefore the liver was considered as the primary site of origin of the tumor.(1) This case represents the first known primary neuroendocrine carcinoma of the liver to be reported in a non-human primate.(1-3) There are rare reports of primary hepatic neuroendocrine carcinomas in humans and domestic animals in the literature. As in this case, neuroendocrine carcinomas in humans are consistently immunopositive for chromogranin A, NSE, and synaptophysin.(6) This is contrary to what has been observed in dogs and cats, where the expression of chromogranin A is inconsistent, and NSE and synaptophysin are considered better indicators.(6) The oval cell is considered to be the progeny of the hepatic stem cells and is bipotential in nature, giving rise to both hepatocytes and bile duct cells.(4) These cells are located in the terminal biliary ductules and canal of Hering, which represent the terminal branches of the biliary tree that connects the interhepatocytic bile canaliculi with the biliary ducts in the portal tracts. They express markers of both immature hepatocytes (e.g. α-fetoprotein) and bile ducts cells (e.g. bile duct type cytokeratin).(7) In addition, the hepatic progenitor cell (HPC) compartment has neuro/neuroendocrine features such as the expression of chromogranin A, neural cell adhesion molecule (NCAM), neurotrophin 4/5, neurotrophin receptor tyrosine kinase B and parathyroid hormone related peptide.(4) Roskams *et al.* showed that during the early stages of regeneration, bile duct epithelium displays neuroendocrine features including cytoplasmic, dense core neurosecretory granules and chromogranin-A expression while reactive bile ductules have been shown to express NSE.(7)

AFIP Diagnosis: Liver: Neuroendocrine carcinoma, low grade (carcinoid).

Conference Comment: The contributor has provided an excellent example, accompanied by an informative overview, of a rare entity. Conference participants contemplated a diagnosis of adenocarcinoma based on the formation of cystic and tubular structures by neoplastic cells, but uniformly favored the contributor's diagnosis because of the prominent rosette-like glandular structures that have basally-situated nuclei and finely granular cytoplasm, characteristic of neuroendocrine carcinoma. Because of potential prognostic implications, correctly distinguishing hepatic neuroendocrine carcinoma from biliary adenocarcinoma or hepatocellular carcinoma is of paramount importance. For instance, in one study, dogs with hepatic neuroendocrine carcinoma experienced a significantly shorter survival/euthanasia interval than did those with biliary adenocarcinoma or hepatocellular carcinoma. In dogs and cats, cytokeratin AE1/AE3 immunostaining is reportedly positive in adenocarcinoma and negative in hepatic neuroendocrine carcinoma, testifying to the utility of immunohistochemistry in making the distinction.(6) In the present case, results of the immunohistochemical stains listed by the contributor and repeated at the AFIP confirmed the diagnosis of neuroendocrine carcinoma.

Conference attendees briefly reviewed the term "carcinoid," which is well-ensconced in the texts, where it is sometimes used interchangeably with neuroendocrine carcinoma. Although the term is used less frequently in the

recent literature, it facilitates professional communication, because unlike "neuroendocrine carcinoma," it requires no further modification to connote low-grade malignancy. In humans, carcinoids are most frequently identified in the gastrointestinal tract, followed by the tracheobronchial tree and lungs. Among gastrointestinal carcinoids, those of the jejunum and ileum are most common, followed by those of the colorectum and appendix; those of the stomach, proximal duodenum, and esophagus are least common. Gastrointestinal carcinoids arise from cells that release peptide and nonpeptide hormones to coordinate gastrointestinal function; these cells, formerly referred to as amine precursor uptake and decarboxylation (APUD) cells, comprise the diffuse endocrine system of the gastrointestinal tract. In humans, carcinoid syndrome is a rare clinical manifestation of the neoplasm characterized by cutaneous flushing, sweating, bronchospasm, abdominal pain, diarrhea, and fibrosis of right-sided heart valves. The syndrome results from the secretion and systemic release of vasoactive substances by the tumor, and is strongly associated with metastatic disease.(8) While carcinoid syndrome has not been reported in animals, conference attendees discussed neuroendocrine tumor-related paraneoplastic syndromes documented in veterinary medicine, such as Zollinger-Ellison syndrome due to functional gastrin-secreting tumors, and superficial necrolytic dermatitis, which is sometimes associated with glucagonomas.

This case was reviewed in consultation with the Departments of Hepatic and Soft Tissue Pathology at the AFIP, both of which concurred with the above diagnosis, while emphasizing the importance of excluding the possibility of hepatic metastasis from a primary gastrointestinal carcinoid because of the extreme rarity of primary hepatic neuroendocrine carcinoma.

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References:

1. Aloisio F, Dick EJ Jr, Hubbard GB: Primary hepatic neuroendocrine carcinoma in a baboon (*Papio* sp.). J Med Primatol **38:**23-26, 2009
2. Cianciolo RE, Butler SD, Eggers JS, Dick EJ, Leland M, de la Garza M, Brasky KM, Cummins LB, Hubbard GB: Spontaneous neoplasia in the baboon (*Papio* spp.). J Med Primatol **36:**61-79, 2007
3. Cianciolo RE, Hubbard GB: A review of spontaneous neoplasia in baboons (*Papio* spp.). J Med Primatol **34:**51-66, 2005
4. Libbrecht L, Roskams T: Hepatic progenitor cells in human liver diseases. Semin Cell Dev Biol **13:**389-396, 2002
5. Patnaik AK, Lieberman PH, Erlandson RA, Antonescu C: Hepatobiliary neuroendocrine carcinoma in cats: a clinicopathologic, immunohistochemical, and ultrastructural study of 17 cases. Vet Pathol **42:**331-337, 2005
6. Patnaik AK, Newman SJ, Scase T, Erlandson RA, Antonescu C, Craft D, Bergman PJ: Canine hepatic neuroendocrine carcinoma: an immunohistochemical and electron microscopic study. Vet Pathol **42:**140-146, 2005
7. Roskams T, Cassiman D, De Vos R, Libbrecht L: Neuroregulation of the neuroendocrine compartment of the liver. Anat Rec A Discov Mol Cell Evol Biol **280:**910-23, 2004
8. Turner JR: The gastrointestinal tract. *In:* Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, Aster JC, 8th ed., pp. 787-789. Saunders Elsevier, Philadelphia, PA, 2010

CASE IV: D0810008 (AFIP 3149417).

Signalment: 1.5 year-old, female, Angus cow (*Bos taurus*).

History: This heifer was one of 200 head of Angus beef cattle that were rotated through a new pasture for 2 days then moved back to previous pasture. Initially, 3 were found dead, and 3 sick cows, presenting weak and anorexic, were hospitalized. The number of sick cows increased to 30, and 15 of these were reported dead within 3 days. Two cows survived at the hospital and became icteric, with elevated liver enzymes.

Gross Pathology: At necropsy, the heifer was in good muscular condition. The liver was uniformly swollen and pale, and on cut sections an enhanced reticular pattern was evident, somewhat resembling a nutmeg liver. Numerous petechiae were noted within the epicardium and endocardium, but the hemorrhages did not extend into the subjacent myocardium.

Laboratory Results: Microcystin LR was detected in the rumen and cecal contents by liquid chromatography-mass spectrometry.

Histopathologic Description: Liver: There is severe periacinar to massive hepatic necrosis. Periportal hepatocytes are often spared, and form a bridging border around the necrotic foci. The hepatocytes are disassociated from their neighboring hepatocytes, and have rounded up. There is moderate sinusoidal hemorrhage within the fields of the disassociated hepatocytes. Sloughed cells admixed with necrotic debris and inflammatory cells accumulate within the central vein lumens.

Contributor's Morphologic Diagnosis: Liver: Hepatitis, periacinar to massive, acute, necrotizing, with hepatocytic dissociation and sinusoidal hemorrhage.

Contributor's Comment: Microcystin LR (MCLR) is a microcyclic heptapeptide hepatotoxin, a secondary metabolite that is produced during excessive growth and bloom of cyanobacterium (blue-green algae) in water sources. Cyanobacterial bloom occurs most often in water that contains high concentrations of mineral nutrients, especially phosphorus, and during times of sustained sun exposure with calm wind conditions.(3) After production, cyanotoxins are often blown to the shallow regions and margins of the water source, effectively concentrating their levels.

After ingestion of water contaminated with MCLR, the toxin is rapidly taken up in hepatocytes by carrier-mediated transport. Within the hepatocytes, MCLR inhibits protein phosphorylases 1 and 2A, which regulate cellular structural proteins. Inhibition of phosphorylases 1 and 2A promotes hyperphosphorylation of cytoskeletal proteins. Hepatocyte and endothelial cytoskeletal actin filaments distort and collapse, causing cellular structure alteration, detachment from adjoining hepatocytes and membrane instability.(1,2)

In early stages, periacinar to massive hepatic necrosis is represented by the detachment and rounding up of the altered hepatocytes and endothelial cells, extrusion of blood into the disrupted sinuses, with blood and necrotic, fully detached hepatocytes flowing into the central venous network. Hypovolemic shock due to hepatic hemorrhage usually causes death, although hypoglycemia and hyperkalemia from fulminant cessation of hepatic function can also be terminal events.(3) In cumulative low doses, liver failure, chronic inflammation and bilirubinemia can result in mortality.(3) If the animal survives the initial episode of hepatic damage, leukocytic inflammation within the periacinar necrosis proceeds to postnecrotic scarring, periacinar fibrosis, and irregular areas of parenchymal regeneration.(4)

Microcystin LR was detected in the rumen contents from this heifer, and additional heifers that died following exposure. Acute hepatic necrosis with hemorrhage was featured in the early cases that were submitted, and heifers that survived and later died exhibited subacute microscopic lesions. This incident occurred during late summer in Northern California, and site visits to the pasture where the affected heifers were housed found one water source that was a shallow, warm pond with brackish water and blue-green algae growth.

AFIP Diagnosis: Liver: Necrosis, centrilobular to midzonal, diffuse, with hepatocellular dissociation, hemorrhage and biliary hyperplasia.

Conference Comment: Participants were approximately evenly divided between morphologic diagnoses of necrotizing hepatitis and hepatocellular necrosis, and debated the merits of each during the conference session, ultimately favoring the latter because of the absence of inflammation in the sections available for examination. Massive hepatic necrosis indicates necrosis of entire acini and is present in some areas of the examined sections. Submassive necrosis, with sparing of periportal hepatocytes, is prevalent. Using the acinar approach to hepatic histology, the findings could be summarized as submassive necrosis involving acinar zones 2 and 3.

This case was also studied in consultation with the AFIP Department of Hepatic Pathology; their differential diagnosis for the histologic lesions was broad, and included viral infection, severe ischemia, metabolic disease, and acute autoimmune hepatitis, but a toxic etiology was favored. As detailed in the conference proceedings for WSC 2009-2010, Conference 16, case III, numerous plant toxins – some of which are summarized in a table therein – preferentially target centrilobular hepatocytes because of the extraordinary vulnerability of this cell population to hypoxia and their relatively high concentration of cytochromes P450. Therefore, the recognition of a centrilobular to midzonal distribution of hepatocellular necrosis, while essential to the development of an inclusive differential diagnosis, is not sufficient to definitively implicate a particular plant toxin. Additional evidence is required, and in this case, prominent hepatocellular dissociation is a key clue to prompt further investigation of MCLR as a possible etiology. Reiterating the importance of ancillary diagnostic tests, particularly in cases of suspected toxicosis, the detection of MCLR in ruminal and cecal contents by liquid chromatography-mass spectrometry solidifies the diagnosis in this case. The contributor provides an edifying review of the entity.

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References:

1. Batista T, deSousa G, Suput JS, Rahmani R, Suput D: Microcystin-LR causes the collapse of actin filaments in primary human hepatocytes. Aquat Toxicol **65:**85-91, 2003
2. Billiam M, Mukhi S, Tang L, Gao W, Wang JS: Toxic response indicators of microcystin-LR in F344 rats following a single-dose treatment. Toxicon **51:**1068-1080, 2008
3. Briand JF, Jacquet S, Bernard C, Humbert JF: Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. Vet Res **34:**361-377, 2003
4. Stalker MJ, Hayes MA: Liver and biliary system. *In:* Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 2, p. 327. Saunders Elsevier, Philadelphia, PA, 2007