

Case Report

J Vet Intern Med 2016;30:1747–1751**Presumptive Iatrogenic Microcystin-Associated Liver Failure and Encephalopathy in a Holsteiner Gelding**

N.S. Mittelman, J.B. Engiles, L. Murphy, D. Vudathala, and A.L. Johnson

An 8-year-old Holsteiner gelding was presented for evaluation of anorexia, obtundation, icterus, and mild colic signs of 48 hours duration. History, physical examination, and initial diagnostics were suggestive of hepatic disease and encephalopathy. Microcystin toxicosis was suspected based on historical administration of a cyanobacteria supplement, associated serum biochemistry abnormalities, and characteristic histopathological changes. Microcystin contamination was confirmed in both supplement containers fed to the horse. Fulminant hepatic failure and encephalopathy progressed resulting in euthanasia. Necropsy findings were consistent with microcystin induced liver failure.

Key words: Bile acids; Horse; Hyponatremia; Liver; Necrosis.

An 8-year-old 569-kg Holsteiner gelding was examined for anorexia, depression, icterus, and mild colic signs of 48-hour duration. The gelding received monthly allergen immunotherapy and had received a rhinopneumonitis vaccine^a three days before onset of clinical signs. The horse had travelled to a horse show within Pennsylvania three weeks before presentation. None of the other 30 horses on the farm had any signs of illness. The horse's diet consisted of timothy hay, a commercial ration-balancing feed,^{b,c} and grass pasture at night. The horse had also received a commercial powdered blue-green algae supplement^d for purported hoof health benefits for 2 months, of which a new container was used three days before presentation. Automatic waterers were cleaned daily. Anthelmintic treatment was based on routine fecal egg counts.

On presentation, the gelding was obtunded and yawned continuously, but displayed no other neurologic abnormalities. Body condition was 5/9 with a smooth hair coat with no signs of photosensitization on its white markings. Rectal temperature was 99.9°F, heart rate was 40 beats/min, and respiratory rate was

Abbreviation:

MC microcystin

20 breaths/min with normal respiratory effort. Marked icterus was detected on examination of the conjunctiva and oral mucous membranes. Capillary refill time was <2 seconds with no petechiae or ecchymoses. Gastrointestinal borborygmi were decreased but present in all quadrants. No other abnormalities were detected on physical examination, abdominal palpation per rectum, or sonographic examination of the abdomen. Nasogastric intubation yielded no net reflux.

Abnormalities were not detected on CBC. Plasma fibrinogen concentration was within normal limits (217 mg/dL; ref range 200–400 mg/dL). Biochemical abnormalities included increases in concentrations of: triglycerides (174 mg/dL; ref range 11–52 mg/dL), total protein (7.6 g/dL; ref range 4.6–6.9 g/dL), lactate (19 mg/dL; ref range 6.3–15.3 mg/dL), and serum activity of SDH (10.25 U/L; ref range 0.3–7.0 U/L), AST (1402 U/L; ref range 205–555 U/L), ammonia (245 µg/dL ref range:<60 µg/dL), GGT (184 U/L; ref range 12–45 U/L), and concentrations of bile acids (44 mg/L, ref range 0.39–3.4 mg/L), total bilirubin (14.8 mg/dL; ref range 0.1–1.9 mg/dL), indirect bilirubin (13.2 mg/dL; ref range 0.1–1.6 mg/dL), and direct bilirubin (1.6 mg/dL; ref range 0.0–0.3 mg/dL). Urinalysis revealed bilirubinuria and urine specific gravity of 1.030.

Medical management was instituted to prevent hyperammonemia, reduce inflammation and provide hepatic support and included: lactulose^e (0.5 mL/kg via nasogastric tube q6h), metronidazole^f (15 mg/kg PO q6h), enrofloxacin^g (5 mg/kg IV q24h), flunixin meglumine^e (1.1 mg/kg IV q12h), pentoxifylline^h (10 mg/kg PO with suspension vehicleⁱ q12h, and S-adenosylmethionine (SAME)^j (20 mg/kg PO q24h). Balanced polyionic crystalloid fluids^k were administered IV as a bolus (20 mL/kg) followed by continuous infusion (4 mL/kg/h) supplemented with 20 meq KCL/L and 2.5% dextrose. Mannitol^l (20% solution; 1 g/kg IV over 30 minutes) was administered to decrease potential cerebral edema because of hepatoencephalopathy.

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Twenty-four hours after admission, ammonia had decreased to 66 $\mu\text{g/dL}$ (ref range $<60 \mu\text{g/dL}$). Prothrombin time (PT; 13.6 seconds ref range 9–12 seconds) and activated partial thromboplastin time (APTT; 50.0 seconds; ref range 34–55 seconds) were mildly elevated and within normal limits, respectively. Abdominal ultrasonography revealed a gas-distended stomach extending from the 9th to 15th intercostal space (ref range 9th–13th¹) and displacing the lungs 25 cm dorsally. Sonographically, the liver was small with normal echogenicity and no evidence of cholelithiasis or cholecystolithiasis. Two core liver biopsies were obtained percutaneously under ultrasound guidance using a 14-g core biopsy instrument^m in the 14th intercostal space. No biopsy complications occurred. Histology revealed predominantly periportal to midzonal hepatocellular necrosis characterized by cytoplasmic hypereosinophilia and shrinkage with pyknosis and karyorrhexis. Bile ducts were unaffected, fibrosis was not present, and inflammation was minimal including periportal lymphocytes and macrophages and rare pigment granulomas. The liver changes suggested a severe acute to subacute toxic change and was unusual in that hepatocytes from all zones of the liver were damaged with no preference for centrilobular hepatocytes. Anaerobic culture yielded no growth. *Staphylococcus sp.* was present in the broth only in the aerobic culture and was considered a contaminant.

Two days later, repeated biochemistry and liver panel revealed increasing concentrations of bile acids (53 mg/L; ref range 0.39–3.4 mg/L) and direct bilirubin (4.2 mg/dL; ref range 0–0.3 mg/dL) and mild increase in total bilirubin (15.3 mg/dL; ref range 0.1–1.6 mg/dL). SDH activity (5.46 U/L; ref range 0.3–7.0 U/L) returned to within normal limits and AST decreased (960 U/L; ref range 205–555 U/L). The gelding's neurologic condition declined rapidly that evening evident as involuntary biting and compulsive circling to overt ataxia and unresponsive pupillary light reflex (OU), suspected Cushing reflex induced bradycardia, and recumbency. The gelding failed to respond to mannitol^m (1 g/kg IV) and required detomidine hydrochlorideⁿ continuous rate infusion (0.3 $\mu\text{g/kg/min}$) to prevent self-trauma. Repeated biochemistry revealed hyponatremia (124 mEq/L; ref range 132–141 mEq/L) and hypochloremia (84 mEq/L; ref range 94–102 mEq/L). Hypertonic saline^o (7.2%; 4 mL/kg IV bolus) was administered followed by isotonic polyionic crystalloids^f (20 mL/kg bolus), and the horse stood, but maniacal biting continued when the detomidine was discontinued, prompting euthanasia.

On gross postmortem examination, the liver was small (3.1 kg, 0.54% body weight)² and had a flaccid “dish rag” appearance with soft, friable, orange-tan to green-brown parenchyma (Fig 1). Histopathology from three different lobes revealed severe periportal to massive necrosis with sinusoidal dilatation and hemorrhage and relative sparing of centrilobular zones (Fig 2). Higher magnification revealed lobular collapse with multifocal individual hepatocellular necrosis with megalocytosis and polykaryosis (Fig 3). Additional (ie, secondary) changes included early portal–portal bridging fibrosis,

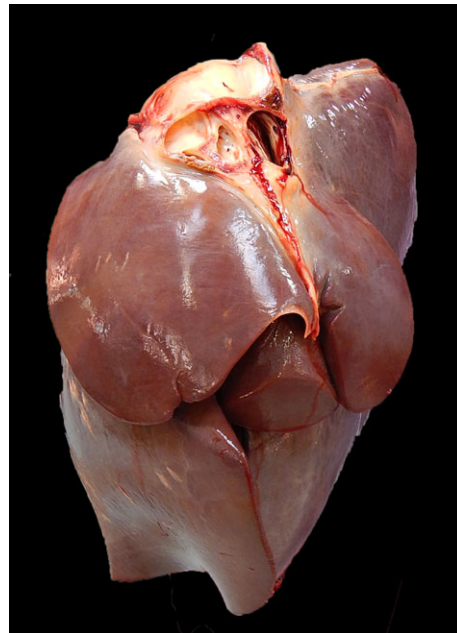


Fig 1. Liver: gross photograph of the limp, flaccid, “dish rag” liver manually suspended after postmortem extraction from the abdomen. The parenchyma is orange-tan to greenish brown, consistent with cholestasis.

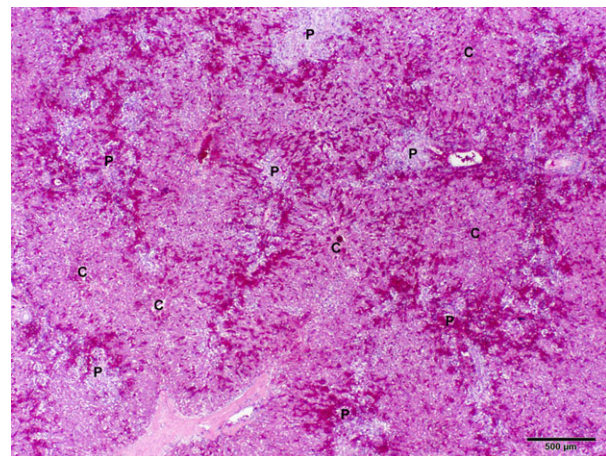


Fig 2. Liver: low-magnification photomicrograph that shows predominant periportal to midzonal necrosis with lobular collapse, sinusoidal dilation, and hemorrhage. Portal (P) and centrilobular (C) zones are labeled. Hematoxylin and eosin stain. Scale bar = 500 μm .

mild bile duct hyperplasia with canalicular cholestasis, and mild inflammation including lymphocytic periportal to midzonal infiltrates with scattered pigment-laden macrophages (Fig 3). Alzheimer type II cells consistent with hepatoencephalopathy were present in the medulla, cerebellum, mesencephalon, diencephalon, and cerebral cortex. Mild eosinophilic colitis was also detected. The stomach was moderately distended with normal ingesta, but was histologically normal. Algal toxin testing (LC/MS) confirmed microcystin in both supplement

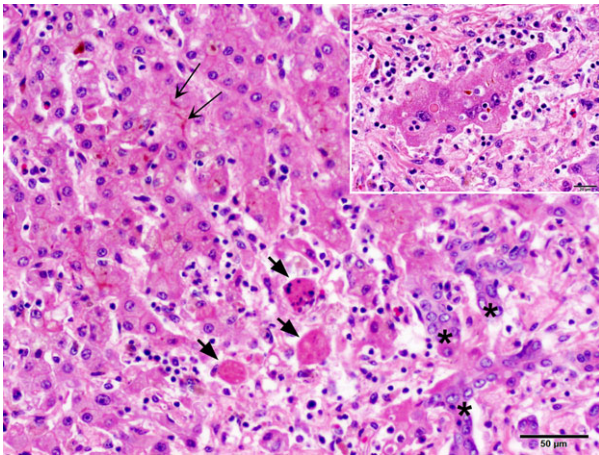


Fig 3. Liver: high-magnification photomicrographs that show periportal individual cell necrosis (thick arrows) with mild bile duct hyperplasia (asterisks), canalicular bile stasis (thin arrows), and lymphocytic infiltrates. Megalocytic and polykaryotic hepatocytes are scattered throughout remaining hepatocytes (inset). Hematoxylin and eosin stain. Scale bar = 50 μm and 100 μm (inset).

containers currently fed in addition to three of five additional containers (0.011–0.5 ppm); however, it could not be isolated from liver, blood, or gastrointestinal contents postmortem. Further organic chemical and mineral screening on stomach contents, liver, and kidney did not reveal any clinically relevant findings.

Discussion

The horse in this report was diagnosed with microcystin-associated hepatic failure and secondary hepatic encephalopathy. Several toxic and infectious causes of acute hepatocellular necrosis and cholestasis were initially considered including microcystin, acute serum hepatitis (Theiler's disease), pyrrolizidine alkaloids, peritonitis, bacterial cholangiohepatitis with or without cholelithiasis, chronic active hepatitis, hemochromatosis, Fall Panicum (*Panicum dichotomiflorum*) toxicosis, Alsike clover, mycotoxins (eg, aflatoxicosis, fusarium, and moldy alfalfa), or poisonous mushrooms (eg, *Amanita verna*). However, anamnesis, histology, and toxicology strongly implicated microcystin intoxication from consuming a blue-green algae supplement.

Blue-green algae produce more than 100 congeners of the hepatotoxic microcystin (MC).^{3,4} MC-LR is the most common congener and has caused liver necrosis in humans, mice, rats, guinea pigs, sheep, cattle, and dogs.^{5–9} Most microcystin toxicoses result from contaminated water consumption; however, important exceptions exist including recent water contamination in a dialysis center that led to twenty-six human deaths from liver failure.^{10,11} A case of hepatic failure in a dog was attributed to high levels of microcystin detected in a *A. flos aquae* supplement harvested from Upper Klamath Lake in Oregon.¹² These algae are harvested during algal blooms in an open environment

making inadvertent collection of *M. aeruginosa*, a microcystin-producing blue-green algae, and microcystin a real concern. In 1996, 85 of 87 blue-green algae supplements derived from this lake were contaminated with microcystin and 72% of those samples exceeded the Oregon Health Department 1 $\mu\text{g/g}$ limit (reaching up to 16.4 $\mu\text{g/g}$),¹³ a limit based on the World Health Organization (WHO) recommendation on drinking water for people.¹⁴ Supplement safety concern is compounded by the ability of *A. flos aquae* to produce a multitude of other potentially lethal toxins including anatoxin-a, saxitoxin, cylindrospermopsin, and BMAA22.¹⁵

Histology supported microcystin intoxication over other causes. Toxic by-products of cytochrome P450 metabolism commonly cause centrilobular necrosis, but periportal necrosis is rare and due to direct hepatotoxicity, as can be seen with microcystin. Microcystin is absorbed preferentially in the ileum, enters portal circulation, and is rapidly cleared from the blood.^{16,17} The toxin is not cell permeant and carrier-mediated transport leads to hepatocyte accumulation.^{18,19} In hepatocytes the toxin binds to and inhibits serine/threonine phosphatases type 1 and 2A leading to hyperphosphorylation and disassembly of cytoskeletal proteins including intermediate filaments, actin microtubules, and actin microfilaments.^{20–22} Acute intoxication causes membrane blebbing, rounding, and loss of cell-to-cell adhesion, leading to hepatic structure breakdown. Hepatic sinusoids collapse, and massive intrahepatic hemorrhage and death ensue.²⁰ Chronic intoxication can lead to tumor promotion because of hyperphosphorylation of cytokeratins via the same phosphatase inhibition.²³ The only other description of similar periportal necrosis in a horse is a case report of a horse with fatal hepatic necrosis after ingesting *Amanita verna*.²⁴ No historical poisonous mushroom exposure or characteristic histologic lesions (eg, nuclear vesiculation and nucleolar loss or fragmentation) consistent with amanitotoxins makes this etiology unlikely. Iron toxicosis, pyrrolizidine alkaloids or Fall Panicum were excluded because of anamnesis and lack of characteristic histologic findings (eg, hemochromatosis or characteristic biliary lesions). No heavy metals or organic chemicals were identified within liver or kidney.

Detection of microcystin in the blue-green algae supplements consumed by the horse also supports this toxin as the cause of disease. It is difficult to confirm whether the horse consumed a lethal dose for several reasons. No established microcystin toxicity data exists in horses and there is wide species variation in toxicity depending on species affected and route of administration (eg, oral LD₅₀ for MC-LR in mice is 10.9 mg/kg versus the intraperitoneal LD₅₀ of 43 $\mu\text{g/kg}$).^{25,26} Whereas it is known that MC-LA and MC-YR have a similar LD₅₀ range as MC-LR, no studies have documented whether there is a linear additive effect or whether toxicokinetics change when other congeners are present, and it is possible that depending on the testing method not all congeners are even detected.²⁷ The WHO has set the tolerable daily intake for human

ingestion of MC-LR at 0.04 µg/kg/d.²⁸ Based on measured levels, the gelding is estimated to have ingested approximately 3 µg of microcystins daily. However, the range in microcystin levels detected in the supplements shows a lack of homogeneity in contamination, and hepatotoxicity may have resulted from ingestion of larger amounts of microcystin unevenly distributed in the supplement. Lack of microcystin detection from liver, blood, and gastrointestinal contents postmortem may be a function of rapid toxin metabolism or lack of test sensitivity or specificity for microcystin congeners. Stomach contents gathered at the time of consumption and not three days later might have been a better diagnostic specimen for microcystin testing.

The most important differential diagnosis for this case was Theiler's disease, which can also result in acute fulminant hepatic failure, encephalopathy, and death within days. The small "dish rag" liver because of cytoskeletal loss and massive necrosis is a characteristic but not pathognomonic finding in Theiler's disease. The histologic pattern of necrosis in Theiler's disease, however, is predominantly centrilobular without the megalocytosis/karyosis and polykaryosis seen in this case.²⁹ Epidemiologic findings vary from adult horses receiving equine origin blood products (eg, tetanus antitoxin or hyperimmune plasma) 1–3 months earlier to horses in contact with those treated, or no exposure to either.³⁰ Recent research suggests an underlying viral etiology.

Gastroparesis, evident in this case by a distended stomach sonographically, might be a negative prognostic indicator. Delayed gastric emptying is associated with cirrhosis in human,³¹ and gastric impaction has been reported secondary to pyrrolizidine alkaloid ingestion in ponies.³² Abnormal gastric motility might result from enteric neuronal dysfunction because of the same toxins that cause cerebral signs of hepatic encephalopathy. In 50 cases of primary equine hepatic disease 7 of 10 horses with hepatic encephalopathy had gastric impactions, and none survived.³³ Hyponatremia in fulminant liver failure is another unusual case feature and might not be because of mannitol-induced dilutional hyponatremia. Acute liver failure alone can result in third space losses into the gastrointestinal tract and peritoneal cavity and blood pooling in the portal circulation. In human cirrhosis, hyponatremia occurs secondary to overall reduced effective blood volume and reduced renal perfusion leading to nonosmotic hypersecretion of vasopressin because of arterial under-filling.³⁴ Similar mechanisms of hyponatremia were suspected as transient portal hypertension, and decreased hepatic and renal perfusion has been reported in microcystin-LR administration to pigs.¹⁷ Concurrent hyponatremia could be an important prognostic finding in equine liver failure; its presence in human liver failure is strongly correlated with increased risk of encephalopathy and death.³⁵

Equine deaths attributed to algal blooms date back to 1878,³⁶ but the recent use of blue-green algae

(*Aphanizomenon flos aquae*) supplements in people and animals necessitate its reconsideration as a cause of hepatic failure and death. This case illustrates the clinicopathological and postmortem findings in a gelding that consumed a commercial *A. flos aquae* supplement and was euthanized because of hepatic failure consistent with microcystin intoxication. Concurrent hyponatremia in liver failure is a poor prognostic indicator for survival in people and requires more investigation in veterinary species. In cases of liver failure in horses, the importance of a thorough medication and dietary history is of paramount importance.

Footnotes

- ^a Pneumabort-K[®] + 1b Zoetis Inc., Kalamazoo, MI 49007
 - ^b ENRICH PLUS[®] 2013 Purina Animal Nutrition, LLC
 - ^c E3 Live for horses ©2014 Distributed by: G.A.L.E. Inc, DBA E3 LIVE[™]FOR HORSES, DBA The Perfect Horse[™]
 - ^d FLUNIXIJECT (flunixin meglumine) Injectable Solution Henry Schein Animal Health Dublin, OH 43017
 - ^e Plasma-Lyte A Injection pH 7.4 (Multiple Electrolytes Injection Type 1 USP) 1000 mL Baxter Healthcare Corporation Deerfield IL 60015
 - ^f Constulose Lactulose Solution USP (10 g/15 mL) Actavis Inc. 60 Columbia Rd Bldg B Morristown, NJ 07960
 - ^g Metronidazole Tablets USP Heritage Pharmaceuticals Inc. Eatontown, NJ 07724
 - ^h Baytril[®]100 Bayer Healthcare LLC. Animal Healthcare LLC. , Animal Health Division Shawnee Mission, Kansas 66201
 - ⁱ Pentoxifylline Extended-Release Tablets, USP 400 mg Apotex Inc. Toronto, Ontario Canada M9L1T9
 - ^j ORA-PLUS[™] Paddock Laboratories, INC. 3940 Quebec Avenue North Minneapolis, Minnesota
 - ^k SAME 400 mg NOW FOODS 395 S. Glen Elly Rd., Bloomington, IL 60108
 - ^l Mannitol Injection, USP (20 g/100 mL) Hospira Inc., Lake Forest IL 60045
 - ^m BARD[®] MONOPTY[®] Instrument C. R. Bard, Inc. Covington, GA 30014
 - ⁿ Dormosedan[®] Zoetis Inc., Kalamazoo, MI 49007
 - ^o Hypertonic Saline Solution 8× Butler Schein Animal Health Dublin, OH 43017
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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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