Liver Failure in a Dog Following Suspected Ingestion of Blue-Green Algae (*Microcystis* spp.): A Case Report and Review of the Toxin

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ABSTRACT

A 2.5 yr old spayed female Weimaraner presented after ingestion of blue-green algae (*Microcystis* spp.). One day prior to presentation, the patient was swimming at a local lake known to be contaminated with high levels of blue-green algae that was responsible for deaths of several other dogs the same summer. The patient presented 24 hr after exposure with vomiting, inappetence, weakness, and lethargy. Blood work at the time of admission was consistent with acute hepatic failure, characteristic findings of intoxication by *Microcystis* spp. Diagnosis was suspected by analyzing a water sample from the location where the patient was swimming. Supportive care including fluids, fresh frozen plasma, whole blood, vitamin K, B complex vitamins, *S*-adenosyl methionine, and *Silybum marianum* were started. The patient was discharged on supportive medications, and follow-up blood work showed continued improvement. Ingestion is typically fatal for most patients. This is the first canine to be reported in the literature to survive treatment after known exposure. (*J Am Anim Hosp Assoc* 2013; 49:342–346. DOI 10.5326/JAAHA-MS-5913)

Introduction

Cyanobacteria, also known as blue-green algae, are a family of prokaryotes that proliferate in water bodies such as ponds, lakes, reservoirs, and slow-moving streams when the water is warm and nutrients are available.[1] Mammal poisoning associated with toxic blooms of cyanobacteria have been reported worldwide. The mechanisms of toxicity for cyanotoxins are very diverse, including toxic effects on the liver, nervous system, kidneys, gastrointestinal tract, respiratory tract, and skin. Three major toxins have been identified in canine cases: anatoxin-a, microcystin, and nodularin. Only a limited number of microcystin intoxications have been reported in dogs. In those cases, toxicosis is usually either a result of drinking contaminated water or accidental ingestion during swimming.[2,3] The algal material can also adhere to the fur and be ingested when the animal licks its coat. The clinical features, laboratory findings, and therapy associated with a *Microcystis* spp. toxicosis in a dog are reported herein. To the authors' knowledge, there is no other reported case of an intoxicated dog that survived. This report describes presenting clinical signs, clinicopathologic abnormalities, treatment, and follow-up data in a dog after ingestion of *Microcystis* spp.

Case Report

A 2.5 yr old spayed female Weimaraner weighing 24.6 kg presented to the Kansas State Veterinary Health Center for a 1 day history of vomiting, inappetence, weakness, and lethargy. The dog was up-to-date on vaccinations, had no travel history, and no known dietary indiscretion. Twenty-four hr prior to presentation, the dog had swam several hr at Milford Lake, a location known to be contaminated with a high level of toxic blue-green algae by the Kansas Department of Health and Environment and confirmed to have caused the death of several dogs the same summer.[4,5] The owners reported the dog had ingested a significant amount of water and was covered with green material. She was

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ALP *alkaline phosphatase;* ALT *alanine aminotransferase;* APTT *activated partial thromboplastin time;* PO *per os;* PT *prothrombin time;* SC *subcutaneously*

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bathed after returning home, but several hours later became weak, inappetent, and started vomiting clear and foamy material. Throughout the night and the next day, vomiting occurred every few min to hr, and the patient became progressively lethargic.

At the time of presentation, the patient was weak, approximately 7% dehydrated, and had fair femoral pulse quality. The mucous membranes were dry, slightly pale, and had a capillary refill time of 2 sec. The cranial abdomen was moderately painful on palpation.

Samples were submitted for a complete blood count, a serum biochemical profile, and a coagulation panel (i.e., prothrombin time [PT], activated partial thromboplastin time [APTT]). Results of the complete blood count revealed a mild erythrocytosis (8.61×10^{12}/L; reference range, 5.5–8.5 $\times 10^{12}$/L), moderate neutrophilia (14.4×10^{9}/L; reference range, 3–11.5 $\times 10^{9}$/L), moderate lymphopenia (0.037 [proportion of 1.0]; reference range, 1.5–5 $\times 10^{9}$/L), and moderate thrombocytopenia ($61 \times 10^{9/}$L; reference range, 164–510 $\times 10^{9}$/L). The abnormalities on the biochemical profile included a severe increase in alanine aminotransferase ([ALT]; 1,011.77 μkat/L; reference range, 0.47–2.86 μkat/L), moderate increase in alkaline phosphatase ([ALP]; 4.59 μkat/L; reference range, 0.0167–2.37 μkat/L), creatine kinase (22.38 μkat/L; reference range, 2.14–5.48 μkat/L), hyperphosphatemia (2.23 mmol/L; reference range, 0.78–2.07 mmol/L), hyperbilirubinemia (59.85 μmol/L; reference range, 1.71–5.13 μmol/L), mild hypokalemia (3.5 mmol/L; reference range, 3.6–5.3 mmol/L), mild hypochloremia (106 mmol/L; reference range, 108–118 mmol/L) as summarized in **Table 1**. Blood glucose was within the normal limits (5.66 mmol/L; reference range, 4.05–6.27 mmol/L) as shown in **Table 2**. The coagulation panel showed a severely prolonged PT (> 100 sec; reference range, 7.1–9.1 sec) and APTT (239.1 sec; reference range, 8.2–12.7 sec) as shown in **Table 3**.

The dog was bathed again with shampoo to remove any remaining algae. Fluid therapy was initiated with sodium chloride 0.9%[a], supplemented with 20 mEq of potassium chloride/L[b]. Initial fluid rate was calculated to correct dehydration over 12 hr while supporting the maintenance requirement and the ongoing losses (approximately 8.3 mL/kg). Vitamin B complex[c] (2 mL/L) was added to the first bag of crystalloids. Vitamin K1[d] (5 mg/kg subcutaneously [SC]), an oral product containing both *S*-adenosyl methionine (18 mg/kg *per os* [PO] *q* 24 hr) and *Silybum marianum* (3 mg/kg PO *q* 24 hr)[e], and a transfusion of fresh frozen plasma (10 mL/kg IV) was started.

A water sample, collected from the location where the dog swam, was submitted for toxicological analysis. The algae were identified as *Microcystis* spp. by light microscopic examination on the basis of morphologic features (**Figure 1**).

On day 2, another coagulation profile, blood glucose, packed cell volume, and serum total protein were performed (Tables 2 and 3). The repeated coagulation profile showed improvements in PT and APTT, although both were still prolonged (65 sec and 71 sec, respectively). Petechiae developed on the dog's ears and abdomen. A second fresh frozen plasma transfusion (10 mL/kg IV) was administered. Supportive care was continued with sodium chloride 0.9% supplemented with 20 mEq of potassium chloride/L, 2.5% dextrose[f] (50 mL of 50%/L), and vitamin B complex (2 mL/L). *S*-adenosyl methionine (18 mg/kg PO *q* 24 hr)/*Silybum marianum* (3 mg/kg PO *q* 24 hr) and vitamin K1 (4 mg/kg SC *q* 8 hr) were also continued. The bruising progressively worsened throughout the day, the total protein decreased to 45 g/L (reference range, 54–76 g/L), and a third fresh frozen plasma transfusion (10 mL/kg IV) was administered. Supportive care continued as previously described, along with maropitant[g] (1 mg/kg SC once) because vomiting had continued intermittently since presentation. The dog remained stable throughout the day; however, the packed cell volume began dropping (Table 2) and a fluid wave in the abdomen was now appreciated on examination.

TABLE 1

Significant Findings on Complete Blood Count and Serum Biochemical Profile

	Reference range	Day 0	Day 3	Day 5	Day 12
WBC count ($\times 10^{9}$/L)	6–17	16	12.7	21.6	12.7
Platelets ($\times 10^{9}$/L)	164–510	61	39	101	217
BUN (mmol/L)	3.21–11.78	8.57	3.21	2.5	8.93
Albumin (g/L)	34–42	41	23	34	37
ALT (μkat/L)	0.47–2.86	1,011.77	251.39	236.20	25.32
ALP (μkat/L)	0.0167–2.35	4.59	3.96	5.14	6.11
Creatine kinase (μkat/L)	2.14–5.48	22.38	2.22	2.36	1.69
Total bilirubin (μmol/L)	1.71–5.13	59.85	186.39	155.61	18.81
Cholesterol (mmol/L)	3.44–10.20	3.65	1.86	3.83	4.33

ALP, alkaline phosphatase; ALT alanine aminotransferase; BUN, blood urea nitrogen.

TABLE 2

Serial Packed Cell Volume, Total Protein, and Blood Glucose Values

	Reference range	Day 0 (AM)	Day 0 (PM)	Day 1 (AM)	Day 1 (PM)	Day 2 (AM)	Day 2 (PM)	Day 3 (AM)	Day 3 (PM)	Day 4 (AM)	Day 4 (PM)	Day 5 (AM)	Day 5 (PM)
Packed cell volume (%)	37–55	61	52	51	38	34	27	25	31	33	37	43	NE
Total protein (g/L)	54–76	70	65	55	45	45	35	37	48	50	59	60	NE
Blood glucose (mmol/L)	4.05-6.27	5.66	5.77	4.72	8.60	7.44	5.83	7.16	NE	6.94	NE	5.77	5.83

NE, Not examined.

On day 3, the patient had become more lethargic, had pale mucous membranes, a distended abdomen, and more petechiae were noted on her ears, limbs, and ventrum. Recheck blood work showed anemia (hematocrit was 21%; reference range, 37–55%), moderate lymphopenia (0.063 [proportion of 1.0]; reference range, 0.8 $\times$ 10^9/L), and severe thrombocytopenia (39 $\times$ 10^9/L). At that time, it was suspected the patient had developed a hemoabdomen; however, neither abdominal ultrasound nor abdominocentesis were performed because the authors wished to prevent additional trauma and bleeding for the patient. The abnormalities on the serum biochemical panel included an increase in ALT (251.39 μkat/L), increase in ALP (3.96 μkat/L), hyperbilirubinemia (186.39 μmol/L), hyperchloremia (124 mmol/L), hypocholesterolemia (1.86 mmol/L; reference range, 3.44–10.20 mmol/L) as shown in Table 1. Blood glucose was mildly elevated (7.16 mmol/L; Table 2). Prolonged PT (30 sec) and prolonged APTT (29 sec) were again noted (Table 3). A whole blood transfusion (20 mL/kg IV) was administered without complication. A blood type was not performed because the patient had no previous transfusion history and blood typing is typically not necessary in dogs prior to the first transfusion. In this case, the donor selected was dog erythrocyte antigen 1.1 negative in an attempt to minimize potential reactions, and was the only available donor at the time of transfusion. The patient had stopped vomiting, and vitamin K therapy was modified to oral dosing (4 mg/kg *q* 8 hr).

On day 4, the patient seemed brighter with neither progression of petechiae nor abdominal distension. At that time, her anorexia was also resolved. Supportive care continued as previously described.

TABLE 3

Serial Coagulation Profiles Obtained During and After Dog's Hospitalization

	Reference range	Day 0	Day 1	Day 3	Day 5	Day 12
PT	7.1–9.1 sec	> 100	65.6	30.2	8.6	7.3
APTT	8.2–12.7 sec	239	71.5	29.4	12.7	10.5

APTT, activated partial thromboplastin time; PT, prothrombin time.

Recheck blood work prior to discharge on day 5 was obtained. Abnormalities included a leukocytosis (21.6 $\times$ 10^9/L; reference range, 6–17 $\times$ 10^9/L), neutrophilia (13.1 $\times$ 10^9/L), monocytosis (4.3 $\times$ 10^9/L; reference range, 0.1–0.8 $\times$ 10^9/L), thrombocytopenia (101 $\times$ 10^9/L), elevated ALT (236.20 μkat/L), elevated ALP (5.14 μkat/L), and hyperbilirubinemia (155.61 μmol/L). The coagulation profile was within normal limits with a PT of 8.6 sec and an APTT of 12.7 sec. The patient was discharged with instruction to continue the *S*-adenosyl methionine/*Silybum marianum* product, vitamin K (4 mg/kg PO *q* 8 hr), ursodiol[h] (12 mg/kg PO *q* 24 hr), and vitamin E[i] (16 IU/kg PO *q* 12 hr).

The patient was re-examined 1 wk after discharge. On physical exam, the dog was mildly icteric. Abnormalities on blood work included a monocytosis (1.2 $\times$ 10^9/L), elevated ALT (25.32 μkat/L), elevated ALP (6.11 μkat/L), and hyperbilirubinemia (18.81 μmol/L; Table 2). The coagulation profile was within normal limits with a PT of 7.3 sec and an APTT of 10.5 sec (Table 3).

Discussion

Background

Cyanobacteria are found in lakes, ponds, rivers, and brackish waters throughout the world. They are an ancient group of prokaryotic organisms that are found in environments as diverse as Antarctic soils and volcanic hot springs, often where no other vegetation can exist.[1] Botanists have originally considered cyanobacteria as "blue-green algae," a term that is still used in the public media. That name derives from the fact that those organisms often contain a specific pigment, phycocyanin, that gives many species a slightly blue-green appearance. The blue pigments enable cyanobacteria to use a wider light spectrum than terrestrial land plants and have provoked their early description as blue-green algae. The photosynthetic apparatus of cyanobacteria is similar to green algae, however, genetically, cyanobacteria must be considered true bacteria, placed within the group *Eubacteria* in the phylogenetic taxonomy.[6]

When conditions are optimal for cyanobacterial replication and growth, the population of cyanobacteria in a water body can

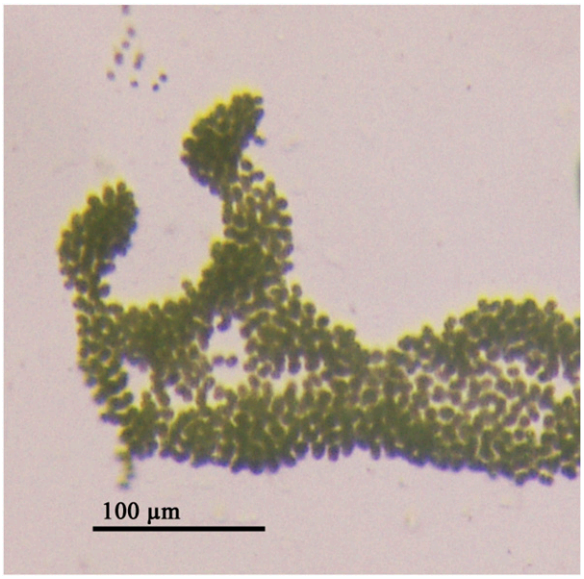

FIGURE 1 *Light microscopic examination of the water sample collected from the lake. Morphologic features are consistent with* Microcystis *spp. Original magnification ×184.*

rapidly build up or "bloom."[1] The onset of bloom formation is determined by a combination of factors, including concentration of nutrients, generally warm temperature, high light density, and stable water and air conditions. Most cyanobacteria float either close to or on the water surface, forming a mass known as algal scum. Dense aggregations of cyanobacterial cells may affect the taste and odor of water supplies. Of greatest concern, however, is the potential of many bloom-forming cyanobacteria to produce a wide range of toxic substances. Those natural compounds, known as cyanotoxins, are chemically diverse and are usually either neurotoxic or hepatotoxic in pathology. Based on their chemical structure, cyanotoxins can be divided into the following three groups: cyclic peptides (microcystins and nodularins), alkaloids (cylindrospermopsins, anatoxins, and saxitoxins) and lipopolysaccharides.[1] The dog described in this report had been intoxicated with microcystins.

Toxicity of Microcystins

An important aspect of pathways of animal exposure is that microcystins do not appear to enter the body through dermal exposure, but chiefly through active transport mechanisms.[7] Consequently, exposure either requires ingestion or aspiration of water or food that contains cyanobacterial cells and/or dissolved cyanotoxins. Microcystin does not readily cross cell membranes and does not enter most tissues. Toxins are absorbed from the small intestine into the liver due to the active uptake by an unspecific organic anion transporter and mainly enter hepatocytes.[7] However, at high concentrations, microcystins may change the barrier properties of cell membranes, leading to enhanced penetration through partitioning that does not require active or facilitated transport.[8]

Microcystins are cyclic heptapeptides that mainly cause hepatotoxicosis.[9] The mammalian toxicity of microcystins is mediated through their strong binding to key cellular enzymes called protein phosphatases. Indeed, microcystins have been found to be potent inhibitors of eukaryotic protein serine/threonine phosphatases 1 and 2A *in vivo*.[10] In liver cells, intermediate filaments of the cytoskeleton are hyperphosphorylated, which result in cell cytoskeletal deformation and bleb formation.[9] Cell lysis and apoptosis follow, depending on the dose. Death results from dissolution of the liver structure and intrahepatic pooling of blood, which can lead to overall cellular disruption and hemorrhagic shock.[11] Other cells (even nonparenchymal liver cells) are much less sensitive to the toxicity of microcystin and require much higher doses and longer incubation times for toxicity.[7,8,12]

Diagnostics

Only a small group of genera and species of cyanobacteria produce toxins that affect animals and humans. Morphologic observation of the algae is not enough to predict the hazard level because toxic and nontoxic strains show no predictable difference in appearance.[1] Therefore, identification of algae material in water and gastric content is an important component of the diagnostic workup, but does not confirm intoxication.

Direct detection of the toxin in gastric content is confirmatory, but such tests are not routinely available at diagnostic laboratories. Although many assays are available to analyze water samples for microcystins, there are only limited methods available to reliably and accurately detect microcystins in biologic specimens collected from animals suspected to have died of microcystins intoxication.[13] Other ways of determining toxicity are by bioassays in mice, other whole animals, or in cells.[14]

Treatment

There is no specific antidote for microcystins. In many cases, the rapid onset of acute hepatotoxicosis does not allow for timely, therapeutic intervention, and mortality rates are very high. In mice, the intraperitoneal administration of rifampin was considered an effective treatment after exposure to micocystin-LR.[15] Rifampin is a membrane-active agent that blocks the bile acid uptake system of hepatocytes. In contrast, many of the other tested compounds, such as glutathione, silymarin, and cyclosporine A, were only beneficial if administered prior to microcystin administration.[16–19] That is important to consider when trying to protect

against microcystin intoxication in chronic exposures. Because microcystins can enhance oxidative stress, antioxidants such as vitamin E and selenium appear to be beneficial, but best protection is likely to be achieved if given prior to microcystin exposure.[19] Activated charcoal is not protective in mice dosed with microcystins.[20]

Glutathione precursors (such as *N*-acetyl cysteine and *S*-adenosyl methionine) can be added to the treatment plan. Indeed, glutathione conjugation with microcystins may represent major detoxification pathways.[21] However, the efficacy of these drugs has not been studied *in vivo*.

Conclusion

Hepatic necrosis due to toxins produced by blue-green algae (cyanobacteria) is a well-known cause of acute hepatic failure and death in wild and domestic animals. Treatment of the hemorrhagic shock and liver insufficiency should include liver protectants, hydrophobic antioxidants (vitamin E, silymarin), IV fluids, glucose, and blood products as needed. To the authors' knowledge, this is the first case report to describe the successful treatment of a patient after exposure to *Microcystis* spp. Owners should still be aware that the prognosis is guarded; however, recovery is also possible if supportive care can be initiated early in the course of the disease.

FOOTNOTES

[a] 0.9% sodium chloride injection USP; Abbott Animal Health, Chicago, IL

[b] Potassium chloride injectable USP; Vedco Inc., St. Joseph, MO

[c] Vitamin B complex 150 injection; Vedco, Inc., St. Joseph, MO

[d] Veda-K1 injection; Vedco Inc., St. Joseph, MO

[e] *S*-Adenosyl-225 (SAMe); Sogeval Laboratories Inc., Irving, TX

[f] Dextrose 50% solution; Vedco, Inc., St. Joseph, MO

[g] Cerenia; Zoetis Inc., Kalamazoo, MI

[h] Usodiol capsules USP; Watson Laboratories, Inc., Parsippany, NJ

[i] Liqui-E; TwinLab, American Fork, UT

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