Swimming in Red Water

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Introduction

Bacillary hemoglobinuria, or Redwater disease, is a worldwide disease that affects mostly cattle but also sheep, goats and rarely other animals such as pigs, horses and elk[10,13]. The disease-causing agent is a gram-positive bacteria found in soil, *Clostridium haemolyticum*, formerly known as *Clostridium novyi* type D, that contains a beta-toxin (phospholipase C) that damages erythrocytes, hepatocytes, and endothelial cells[12]. Although not contagious, it is more common in cattle that are exposed through trematode contaminated pastures[10,11]. In most cases, parasitic hepatic trematodes are a predisposing factor to bacillary hemoglobinuria, creating anaerobic necrosis within the liver, allowing *Clostridium haemolyticum* spores to activate[13]. Clinical signs may not be apparent as this disease progresses rapidly to death in well-conditioned animals. Typical lesions on necropsy include marked icterus, bloody feces, focal to multifocal hepatic necrosis, renal congestion, and hemoglobinuria[10]. Most cattle do not survive[11,13]. Polymerase chain reaction or culture are definitive diagnostic tests to isolate *C. haemolyticum* for the diagnosis of bacillary hemoglobinuria[10,11]. Treatments are limited due to the rapid progression of the disease. Preventions include judicious anthelminthic use against trematodes, rotating cattle to dry pastures, and vaccinating with appropriate clostridial vaccines[7,10].

Signalment/History

"Les Cow 2" is an approximately one-year old angus cross heifer that presented to Mississippi State University College of Veterinary Medicine Diagnostic Laboratory Services for necropsy examination on February 8, 2020. "Les Cow 2" was part of a 32-member herd of cattle. The producer stated she was separated from the herd the morning of presentation and was later found dead with blood diarrhea. A necropsy was performed on another cow of similar

presentation from the same producer earlier in the week. Les Cow 2 was vaccinated with a 7-way clostridial vaccine but the date was unknown.

Gross and Histological Examination

On necropsy examination, "Les Cow 2" had minimal autolysis present. Her body condition was thin. Icterus was present within the sclera and mucus membranes. Once the hide was reflected, yellow, gelatinous subcutaneous adipose tissue was revealed. Visceral adipose tissue was similar in texture and color. There was an increase amount of yellow-tinged, clear fluid within the pericardial sac. The liver revealed multiple areas of dark green linear tracts, fluke exhaust, on external and cut surface. Associated with these tracts were large, flat, ovoid trematode parasites (consistent with *Fascioloides magna*). Also present and associated with these areas was dense fibrous tissue and areas of pale, friable liver parenchyma. The rumen was grossly normal with dry contents. Blood-tinged fluid was present within the small intestines and colon. No formed feces were found throughout the digestive tract. The kidneys were bilaterally diffusely dark red (congestion). The bladder contained dark purple urine. Her brain on gross examination was within normal limits.

Her gross diagnosis included icterus, serous atrophy of subcutaneous and visceral adipose tissue, multifocal severe hepatic necrosis associated with intralesional trematodes, fibrosis and fluke exhaust (iron-porphyrin pigment[2]), marked perirenal edema with hemorrhage, renal vascular congestion, hemorrhagic enteritis, and hemoglobinuria.

The most important microscopic lesions were observed within the liver, kidney and intestines. Microscopic examination of the liver revealed multiple well-demarcated areas of necrosis, characterized by amorphous, eosinophilic areas, adjacent to normal hepatic

parenchyma. Scattered throughout the affected areas were fine granules of dark brown pigment (fluke exhaust) and oval, thin-shelled, operculated trematode type eggs. Periportal regions contained increased amounts of fibroblasts and collagen (fibrosis).

The kidneys exhibited multiple pathogenic changes. Frequently, the cytoplasm of epithelial cells lining the cortical collecting tubules contained dark brown pigment (hemoglobin). The interstitium of the kidney was infiltrated with plasma cells and lymphocytes. Large vessels were congested. Multifocally, apical villi were hypereosinophilic (necrotic) and there were large numbers of extracellular bacilli noted throughout. There were small numbers of plasma cells within the lamina propria. *Eimeria* protozoal organisms were appreciated throughout jejunal sections.

Diagnostics

Cultures (aerobic and anaerobic) of the liver and small intestine revealed aerobic growth of normal enteric flora from the small intestine and anaerobic growth of *Clostridium perfringens* in both the liver and small intestine. A urinalysis confirmed hemoglobinuria. Bovine Viral Diarrhea Virus ELISA testing was performed on an ear notch sample, which was negative. Clostridium species fluorescent antibody (FA) testing was performed within the laboratory which were negative for *C. chauvei, C. novyi, C. septicum,* and *C. sordelleii*. The clostridial organism fluorescent antibody testing was repeated at a different diagnostic laboratory (Texas A&M Veterinary Medical Diagnostic Laboratory). The FA test specified *C. haemolyticum* with *C. novyi* fluorescent antibody testing in addition to the previously mentioned species listed in the MSU-CVM diagnostic panel. This clostridium fluorescent antibody panel was also negative. No other additional testing was performed to confirm the definitive diagnosis for bacillary hemoglobinuria.

Fluorescent antibody (FA) tests are used on tissue smears or cultures using labelled antibodies for identification of organisms[3]. As mentioned previously, a clostridial fluorescent antibody panel was used to isolate *C. haemolyticum*, but was unsuccessful. There are other diagnostics that can be performed for a definitive diagnosis. Immunohistochemistry (IHC) is also a diagnostic test used for the identification of *C. haemolyticum*. The use of both of these diagnostic methods in combination with clinical signs and diagnostic lesion of bacillary hemoglobinuria does not yield a definitive diagnosis due to the reason that these tests use antibodies for *C. novyi*, a closely related species to *C. haemolyticum*[11]. Polymerase chain reaction (PCR) or culture of *C. haemolyticum* are the definitive diagnostic tests for the diagnosis of bacillary hemoglobinuria[10,11]. However, due to the complex requirements for the isolation of *C. haemolyticum* in culture, a presumptive diagnosis based on clinical signs and diagnostic lesions is acceptable[10].

The causative agent responsible for this heifer's hemorrhagic enteritis is *C. perfringens*. Further diagnostics were not conducted to determine the specific type. *C. perfringens* causes enterotoxaemia and hemorrhagic enteritis mostly in younger calves that do not have a well-developed immune system[14]. Other potential causes that contribute to clostridial overgrowth include abrupt increase in starches of feed, stressful events, parasitic disease (such as seen in this cow), and antibiotic therapy[14,8].

Life Cycle and Pathophysiology

There are three significant liver fluke species in the United States that infect ruminants: *Fascioloides magna, Fasciola hepatica*, and *Dicrocoelium dendriticum*[4,6]. *F. magna* and *F. hepatica* are commonly found in multiple regions in the United States, including the Gulf Coast region. *Dicrocoelium dendriticum*, or lancet fluke, is found in the northeastern portion of the

country[6]. An additional liver fluke species, *Fasciola gigantica*, is limited to tropical regions [7]. For the purposes of this case report, the discussion will include only comparisons and contrasts of life cycles of *F. hepatica* and *F. magna*.

F. hepatica is commonly associated with the development of bacillary hemoglobinuria in cattle, however other trematode species have been reported in association, such as *F. magna*[10]. Both *F. hepatica* and *F. magna* have similar life cycles and require an intermediate host, freshwater snails (*Lymnaeid* sp.). Common definitive hosts for *F. magna* are cervids, such as the white-tailed deer[7]. Cattle are aberrant hosts for *F. magna*, therefore they cannot finish their life cycle. *F. magna*, also known as the deer fluke or giant liver fluke, is typically associated in cattle where deer are grazing in the same areas. In contrast, *F. hepatica* does not utilize the deer as a reservoir of infection, but instead cattle contribute to the propagation of their life cycle[4]. Usually, *F. magna* infections are not detrimental to cattle as much as small ruminants[7]. One migrating *F. magna* fluke through the liver can cause death in a small ruminant[6].

Liver fluke eggs leave the definitive host through the bile and into the fecal material. The eggs ideally enter a moist environment and thousands of larval stages (miracidia) emerge from one egg in a period of about four to seven weeks in suitable conditions[9,6]. Suitable conditions for embryonation of the eggs to progress to miracidia depend on regional climate differences, temperature, moisture, and aeration of water[9]. The miracidia swim in aerated water to find and penetrate the intermediate host, a snail[7]. A transformation event occurs from miracidia to sporocyst within the snail and asexual reproduction begins through parthenogenesis[7,9]. Up to two hundred rediae emerge from the sporocysts and migrate throughout the snail, mostly through the hepatobiliary system. The rediae further develop into cercariae, the final larval stage[9]. This maturation process within the snail happens over the course of four to nine weeks[7,9]. The

cercariae leave the snail intermediate host and encyst on grasses and other plant-life[9]. Once they are encysted, they mature into metacercariae, the infective stage, which can last in ideal conditions within the environment from six months to a year[7,6]. These metacercariae are sensitive to extreme cold and will not tolerate dry conditions, which leads to a non-viable metacercariae[7].

The definitive host consumes the metacercariae through grazing in wet fields in the spring, summer, and autumn months, which vary depending on the part of the country[7]. After ingestion, the metacercariae escape the gastrointestinal tract by penetrating through the small intestine and enter the liver parenchyma[6]. Liver flukes are hermaphroditic organisms however they primarily reproduce through cross-fertilization. *F. hepatica* migrate through the liver until they reach the bile ducts where they can reproduce. In deer, *F. magna* migrate through the liver and eventually pair together and cross-fertilization occurs. An immune response is produced from the liver and a pseudocyst is formed around the flukes. Further maturation occurs and fluke eggs exit the pseudocyst and pass into the gastrointestinal tract of the deer and out in feces. Cattle are aberrant hosts and the *F. magna* eggs are unable to penetrate the pseudocyst and remain trapped, discontinuing their life cycle[7]. *F. hepatica* eggs pass into the feces of cattle, which will infect more snails in the environment[4]. The complete life cycle for *F. hepatica* occurs in about 12-16 weeks, which is a shorter time-period compared to the seven-month long prepatent period for *F. magna*. In the Gulf Coast region of the United States where the spring and summer climate is warm and a high amount of rainfall occurs, optimal conditions for transmission of *F. magna* can last up to six months or more[7].

Clostridium haemolyticum (formerly known as *Clostridium novyi* Type D), is an anaerobic, spore-forming, gram positive, motile bacterial rod that is responsible for bacillary hemoglobinuria. *Clostridium* bacteria are spore-forming and are found within the soil and

vegetation of wet areas. Within the environment, the spores are dormant and can remain in a quiescent state until favorable conditions occur, from months to years. The spores are ingested when animals consume the vegetation and are absorbed through the gastrointestinal tract and are phagocytized by macrophages within the liver. These spores can remain dormant within the cytoplasm of the macrophages for long periods of time[11]. Migration from the liver flukes leads to necrosis which creates anaerobic conditions, allowing germination of these spores[10]. *C. haemolyticum* releases a beta toxin phospholipase C, which damages red blood cells, endothelial cells, and hepatic cells through a hydrolysis reaction converting phosphatidylcholine to phosphocholine and diglyceride[11]. This toxin is analogous to the *Clostridium novyi* type B toxin, the agent that causes "Black disease", and there is no disparity between these two toxins[10]. This reaction causes enormous destruction to the animal, including hemolysis, hepatocyte damage, platelet accumulation, and increased vascular permeability. The anaerobic environment is caused by lack of perfusion to the hepatic tissue from thrombosis in the vasculature and hemolysis[11].

Treatment

There are limited treatment options for bacillary hemoglobinuria because of the rapid decline in most cases. In cases of infectious necrotic hepatitis caused by *C. novyi*, penicillin and tetracyclines are effective if given earlier in the course of disease[10]. Takagi et al. experimentally treated a Holstein cow presumptively diagnosed with bacillary hemoglobinuria based on clinical signs with intravenous cefazolin at high doses along with fluid therapy. After several days with no change in clinical signs and laboratory analysis, the antibiotic was switched to combination ampicillin sodium salt and cloxacillin sodium mixture (Vetecillin) and resulted in a complete recovery in the cow[12].

Prevention

Bacillary hemoglobinuria is mainly managed by prevention of the disease. The main ways to prevent bacillary hemoglobinuria are parasite management and clostridial vaccinations. Parasite management involves minimizing exposure to snails, the intermediate host for both *F. hepatica* and *F. magna*. Since the intermediate host flourishes in wet areas, draining pastures and avoiding ponds are recommended. Dead animals within grazing areas should be removed promptly[10].

It is recommended to use anthelminthics judiciously to avoid resistance[10]. Anthelminthics available in the United States that are effective against *F. hepatica* and *F. magna* are clorsulon and albendazole. Treatment schedule depends on the climate and peak transmission period of the area. Cattle are less susceptible to acute fascioliasis from *F. hepatica* than sheep, so it is recommended to only treat cattle when mature stages of *F. hepatica* are developed to reduce production loss (weight gain) and reduce parasite proliferation within the pastures. The recommendation in the southern United States is late summer or early fall. As previously mentioned, cervids are the definitive host for *F. magna*, so eggs are not emitted to the environment through cattle feces. Most bovine livers affected by *F. magna* are eliminated from the human food supply at the time of slaughter. Treating the local cervid population has not been successful and it is nearly impossible to prevent sharing of pastures between cervids and cattle[7].

Vaccination with a *C. haemolyticum* bacterin/toxoid is effective at decreasing the incidence of disease[13]. The recommended vaccine schedule for calves should start at six months of age followed by a booster vaccination three to four weeks later. This immunity is not life-long and vaccines must be administered biannually to cattle in endemic areas[10].

Discussion

In this case report, *Clostridium haemolyticum* was not isolated. Unfortunately, this organism is difficult to grow and isolate due to its extremely strict anaerobic nature[12,11]. *C. perfringens* was the only clostridial species isolated with fluorescent antibody identification methods. It is not uncommon to isolate other clostridial organisms, like *C. perfringens*, when an anaerobic culture is performed[12].

Most polyvalent clostridial vaccines available commercially effectively reduces disease incidence[10]. However, this heifer was vaccinated at an unknown date with a 7-way clostridial vaccine. The 7-way vaccine does not contain *C. haemolyticum*, but it does reduce incidence of disease for other *Clostridial* diseases, such as *C. chauvoeii* (Blackleg), *C. novyi* (Black disease), *C. septicum* (malignant edema), *C. sordellii*, and *C. perfringens* Types C and D. The 8-way clostridium vaccine adds protection *for C. haemolyticum*[5].

Preventing exposure to trematodes may not be feasible in some circumstances. In the early spring in Mississippi, the climate is warm, and it rains often. This causes accumulation of water within pastures without adequate drainage and farmers may not have an additional dry, unexposed area for the cattle to graze.

Differential diagnoses that were considered were enzootic hematuria due to the dark red urine. Leptospirosis, Black disease (*Clostridium novyi* Type B), anaplasmosis, and babesia were also considered because of the presence of icterus[1].

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