Canine Babesiosis

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Clinicopathologic Conference

December 8, 2017

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INTRODUCTION:

Babesiosis is a disease caused by various species of the intraerythrocytic tick-borne protozoan genus *Babesia. Babesia* is considered to be the second most common blood parasite of mammals (1). Babesiosis has a worldwide distribution and impact effecting many species of animals including cattle, horses, sheep, goats, pigs, dogs, and occasionally people. *Babesia* species are characterized by being either large form or small form (2). Canine Babesiosis is commonly vectored by hard ticks and is transmitted to the dog via saliva, however *Babesia* can also be transmitted through blood transfusions and vertically through bite wounds, saliva or ingested blood (3, 4). Vertical transmission is most commonly reported with *Babesia gibsoni* infections in fighting breeds such as Pit Bull Terriers (3).

HISTORY AND PRESENTATION:

John is an approximately 6 year old Pit Bull mix who presented to MSU-CVM Internal Medicine Department for a possible bleeding splenic mass. John had an approximately two month history of lethargy and decreased appetite. John goes on hikes with his owner and his owner noticed that John had began to fatigue more easily the past two hikes. His owner reports John was in a dog fight with a house mate who is also a Pitbull mix one to two months ago. His owner had recently noted a tick on another dog in the house. John is also up to date on vaccines as well as flea, tick and heartworm preventative. John had been inappetent for 24 hours, and his owner noticed that his ears and gums looked pale. John presented to his referring veterinarian on 7/5/17 and was then referred to MSU-CVM Internal Medicine Department due to suspicion of a bleeding splenic mass.

On presentation, John was bright, alert and responsive. His vital parameters were within normal limits (Temperature: 102.7, pulse: 96 beats per minute, respiration: 40 breaths per

minute). Mucous membranes were pale and slightly icteric, however his capillary refill time was normal. He was wagging his tail and very affectionate toward everyone.

PATHOPHYSIOLOGY:

Many different species of *Babesia* effect dogs. In the past, they were categorized into small and large form with small form being *B. gibsoni* and large form being *B. canis* (2). The primary method of differentiation was made by assessing the morphology of the protozoan using microscopy. Today, with the development of molecular methods, the large form of *Babesia* spp. include *B. canis, B. rossi* and *B. vogeli* while the small form includes *B. gibsoni, B. conradae,* and *Theileria annae* (3).

Babesia spp. are transmitted to vertebrate hosts naturally through the bites of various tick species (Table 1) (2).

Table 1

Distribution, vectors, and cytological characteristics of selected *Babesia* spp. that infect dogs.

Species	Geographic distribution	Proven or putative vectors	Cytological appearance
B. rossi	South Africa, Nigeria, Sudan	*Haemophysalis elliptica*	Usually paired
B. canis	Europe	*Dermacentor spp, Rhipicephalus sanguineous*	Usually paired
B. vogeli	Africa, Asia, Europe, North/Central/South America, Australia	*R. sanguineus*	Single or paired
B. gibsoni	Southeast Asia, United States, South America, Australia, Europe	*H. longicornis* *H. bispinosa* *R. sanguineus*	Usually singular
B. conradae	USA (California)	*R. sanguineus*	Ring, tetrade, ameboid

Babesia spp. undergo sexual conjugation and sporogony within the intestinal lumen and haemocoel of the tick. The sporozoites inside the tick's salivary gland are transmitted to the

vertebrate host via a blood meal. Then asexual replication occurs within the erythrocytes and the parasites appear as merozoites (3). In the United States and Australia, the primary mode of transmission for *B. gibsoni* is through bite wounds (2). One study found that dogs with a history of dog fighting are 5.5 times more likely to have *B. gibsoni* than dogs with no history of fighting. In this study, 150 dogs confiscated from a dog fighting operation were compared to 218 dogs randomly selected from shelters with no history of fighting. 34% of the confiscated dogs were PCR positive for *B. gibsoni* while only 0.5% of the dogs with no fighting history were positive. The fighting dogs were negative for all other tick-borne rickettsial diseases (5).

Once inside of the host, in general, *Babesia* spp. cause an intravascular and extravascular hemolytic anemia due to red blood cell lysis by replicating intracellular parasites. Red blood cell destruction occurs because of antibodies binding to the surface of the cell and complement activation, oxidative damage and phagocytosis, and a decreased osmotic fragility. Severe hemolysis leads to hemoglobinemia, hemoglobinuria, bilirubinemia and bilirubinuria (2).

The clinical signs involving *Babesia* spp. infections are variable ranging from subclinical infections to multi-organ failure and death. Common clinical signs for *B. gibsoni* include lymph node enlargement, enlargement of the spleen, small-bowel diarrhea, weight loss, protein-losing nephropathy, polyuria/polydipsia, and abdominal effusion. Bloodwork abnormalities include mild to severe regenerative immune-mediated hemolytic anemia, neutropenia, leukocytosis, hypoalbuminemia, azotemia, and elevation of liver enzymes. *Babesia canis* clinical signs and bloodwork abnormalities include petechiae, epistaxis, vomiting, lymphadenopathy, hypotension, low T3 syndrome, mild to moderate nonregenerative, normochromic and normocytic anemia, leukopenia with neutropenia and/or lymphopenia, hypoalbuminemia, elevation of liver enzymes, and electrolyte abnormalities (3). “Carrier dogs” have been described in Pit Bulls with *B.*

gibsoni. Individuals who survive the acute infection have the potential to become chronic carriers (6). The diagnosis of carrier dogs continues to be a challenge, however PCR assay is the most consistent and reliable test for identification of these individuals (2). False negative PCR results have been reported in chronic babesiosis involving *B. gibsoni* due to parasite elimination from the circulating blood by the host or low levels of circulating organisms (3).

DIAGNOSTIC APPROACH/CONSIDERATIONS:

The standard technique for diagnosis of *Babesia* has been identification of the protozoa in stained blood smears. For cases with moderate to severe parasitemia, this method is reliable, however, there is not a direct correlation between clinical disease and parasite load. Dogs which are chronically infected are difficult to identify with this method due to low and intermittent parasitemia. When identifying large *Babesia,* blood from a capillary such as an ear or nail is the best sample. Furthermore, buffy coat smears may increase diagnostic success (2). Small *Babesia* are difficult to identify under light microscopy and an expert is often needed (3). With a positive or negative blood smear, polymerase chain reaction (PCR) should always be the next diagnostic step (2).

PCR is a sensitive and specific diagnostic technique. It is useful in the identification of dogs with a low parasitemia such as carrier dogs and utilizing DNA to differentiate *Babesia* spp (2). The North Carolina State University Tick-Borne Disease Testing Laboratory or through the Department of Pathobiology at Auburn University currently offers diagnostic testing for PCR analysis (6). Serology such as indirect fluorescent antibody test (IFAT) can identify a previous or current persistent infection. Limitations of serology tests include the possibility of cross reactivity between different *Babesia* spp. and other protozoan parasites (2). Because no single

diagnostic test can identify every case of Babesiosis, it is recommended that a combination of tests is used (7).

Upon physical exam on 7/5/17, John was bright alert and responsive. His vital parameters were within normal limits (Temperature: 102.7, pulse: 96 beats per minute, respiration: 40 breaths per minute). Mucous membranes were pale and slightly icteric, however his capillary refill time was normal. An electrocardiogram, blood pressure, and SpO2 were all within normal limits. FAST scan of John's abdomen and thorax was performed. Abdominal FAST scan revealed an enlarged spleen with no evidence of a mass and no free fluid in the abdomen. Thoracic FAST scan was normal. Blood was drawn for a complete blood count, chemistry, reticulocyte count, heartworm 4Dx, coagulation profile and PCV/TP. Heartworm 4Dx, which tests for heartworm disease, Lyme disease, ehrlichiosis and anaplasmosis, was negative and the coagulation profile was normal. The remainder of the blood work revealed a mildly regenerative hemolytic anemia, mild hyperbilirubinemia, mild hyperproteinemia, and moderate hyperglobulinemia. Blood was drawn for a comprehensive infectious PCR panel. Results from that panel were pending throughout John's hospitalization. A blood smear was made from capillary blood and at this time no protozoa were identified.

7/6/17 abdominal and thoracic radiographs were performed as well as abdominal ultrasound. Imaging revealed a severely enlarged spleen with two small nodules as well as a nodule on the liver. There was no evidence of metastatic neoplasia in the thorax. Fine needle aspirates and cytology of the spleen were obtained revealing no evidence of neoplasia. John had an intermittent fever throughout hospitalization. John's PCV was monitored every 12 hours from 7/5 to 7/13. Although his PCV did decrease as low as 19%, a blood transfusion was never indicated.

TREATMENT AND MANAGEMENT OPTIONS:

For large *Babesia* species, Imidocarb dipropionate at 6.6 mg/kg intramuscularly or subcutaneously is the treatment of choice. Some studies have suggested that a second dose of imidocarb 15 days after the initial injection is appropriate. As for small *Babesia*, the treatment of choice is a combination therapy of atovaquone 13.5 mg/kg orally every 8 hours with fatty food and azithromycin 10 mg/kg orally every 12 hours for 10 days (3). Atovaquone is an antiprotozoal drug that targets the protozoan mitochondrial electron transport inhibiting de novo pyrimidine synthesis. Azithromycin is a macrolide antibiotic that inhibits protein synthesis (8, 3).

Supportive care for Babesia infected patients is often indicated in more severe cases. Intravenous fluid therapy can be initiated if electrolyte abnormalities or dehydration is present. In dogs with clinically affected with anemia, packed red blood cell transfusions should be provided. Plasma transfusions may be needed in cases of disseminated intravascular coagulation or coagulation disorders, and in cases of immune-mediated hemolytic anemia immunosuppressant drugs may be used (3).

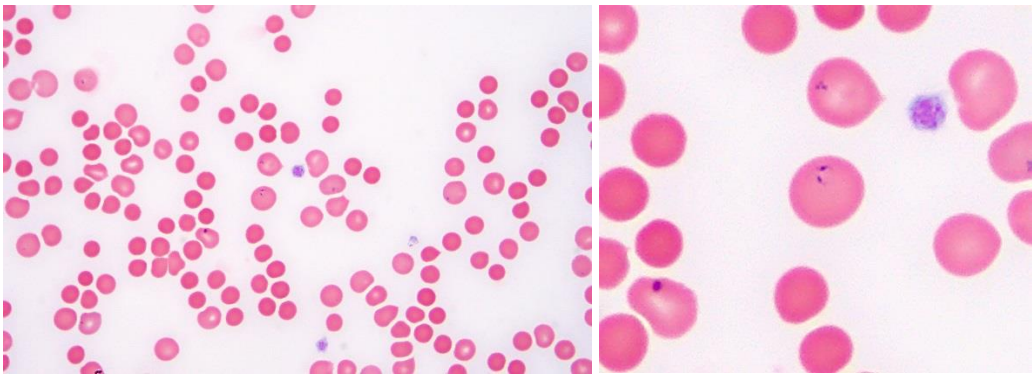
On presentation, it was determined that John was stable and he was started on fluids, Cerenia, and doxycycline. Based on history, clinical signs and bloodwork changes, a presumptive diagnosis was made of *B. gibsoni*. Azithromycin and atovaquone was added to his treatment plan on 7/6.

EXPECTED OUTCOME AND PROGNOSIS:

Most dogs infected with large piroplasms improve in 1-7 days after treatment, although some dogs will not respond until up to 15 days post treatment. After treatment, dogs with large *Babesia* often will make a complete recovery. Poor prognostic indicators for dogs with large

Babesia are moderate anemia, severe thrombocytopenia, mild to moderate leukopenia, hyperlactatemia, moderately increased serum phosphate and triglyceride concentrations and moderately decreased total serum protein concentrations. As for small *Babesia* infections, most dogs who are treated with atovaquone and azithromycin make a complete recovery. In some cases, a persistent infection can remain and when under stress a relapse can occur. This is especially common in splenectomised dogs. It is recommended to perform a PCR 60 days after treatment. This is useful in identifying dogs who remain chronically infected because their chronically infected status predisposes them to relapse (3).

A preliminary PCR result of *Babesia* negative prompted a second look at John's original blood smear. On second look, several small *Babesia* piroplasms were identified and John's atovaquone and azithromycin therapy was continued.



Images provided by Dr. Cooley

On 7/10 John's PCV was 30% and remain there throughout the rest of his hospitalization. John did not have a fever after 7/12/17. At discharge, John was bright and alert and vital parameters were within normal limits (temp: 102.1, pulse: 104 bpm, respiration: 24 brpm). Official PCR results revealed John was positive for *B. gibsoni* Asian genotype and *Mycoplasma hematoparvum*. Doxycycline had been a part of John's therapy which will treat *M.*

hematoparvum. John's owner also had both of John's Pit Bull house mates, whom John had previously been in a fight with, PCR tested. One of his house mates was PCR positive for *B. gibsoni* while the other was not. It is possible that the negative housemate is a chronic carrier with low numbers of circulating organisms. Both housemates where treated with atovaquone and azithromycin therapy.

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