Transfusion-associated *Babesia gibsoni* infection in a dog

Julie R. Stegeman, DVM; Adam J. Birkenheuer, DVM, DACVIM; John M. Kruger, DVM, DACVIM; Edward B. Breitschwerdt, DVM, DACVIM

A 2.5-year-old 29.5-kg (65-lb) spayed female German Shepherd Dog was referred to the Michigan State University Veterinary Teaching Hospital (MSUVTH) with a history of progressive anemia and weight loss after undergoing ovariohysterectomy that had been associated with complications. The dog had been born in Texas but lived its adult life in a breeding facility in Michigan. Prior to adoption by the present owners, the dog had whelped 2 litters. Immediately after ovariohysterectomy, there had been excessive bleeding from the incision; exploratory surgery had revealed generalized oozing of blood from cut surfaces. In the postoperative period, results of a CBC and coagulation profile (including assessment of prothrombin time [PT], partial thromboplastin time [PTT], fibrinogen concentration, and fibrinogen degradation products [FDP] concentration) were within reference limits. The dog had been treated by the referring veterinarian with dexamethasone (3.0 mg/kg, IV, once), vitamin K1 (4.4 mg/kg, SQ, once), IV administration of fluids, and transfusion of fresh whole blood (250 mL) from a clinician. In the postoperative period, results of a CBC and coagulation profile were within reference limits.

Blood samples were obtained, and hematologic abnormalities included moderate regenerative anemia (PCV, 31%; reference range, 43 to 59%; reticulocytes, 150,000 cells/µL; reference range, 60,000 to 135,000 cells/µL) and thrombocytopenia (44,000 platelets/µL; reference range, 192,000 to 534,000 platelets/µL). Erythrocyte morphologic characteristics were unremarkable, and no hemoparasites were observed. A coagulation profile revealed no abnormalities in PT (6.5 seconds; reference range, 6.3 to 8.6 seconds) and fibrinogen concentration (252 mg/dL; reference range, 108 to 287 mg/dL), but there was mildly prolonged PTT (25.8 seconds; reference range, 13.9 to 22.1 seconds), and mildly high FDP concentration (> 5 to < 20 µg/mL; reference range, < 5 µg/mL); a D-dimer assay yielded positive results. Buccal mucosal bleeding time was > 7 minutes (reference range, 2 to 4 minutes). The von Willebrand factor concentration, as measured by ELISA, was 12% of reference value. These findings were consistent with the dog being a von Willebrand carrier; carriers have increased risk of bleeding following surgical or traumatic damage to blood vessels.

Results of serum biochemical analyses indicated moderate hypoalbuminemia (albumin, 2.6 g/dL; reference range, 3.2 to 4.7 g/dL), mild hyperglobulinemia (globulins, 3.2 g/dL; reference range, 2.1 to 2.9 g/dL), and high amylase activity (1,312 U/L; reference range, 415 to 1,175 U/L). A sample of urine was also collected; urine specific gravity was 1.053; urinalysis revealed bilirubinuria (+).
No abnormalities were observed on thoracic radiographs, but abdominal radiography revealed splenomegaly, hepatomegaly, and an area of indistinct soft tissue radiopacity in the mid-caudal abdomen that caused the colon to deviate slightly dorsally. Abdominal ultrasonography revealed a hypoechoic, irregular, and indistinctly margined tubular structure dorsal to the urinary bladder, which was consistent with the uterine stump. The liver and spleen were enlarged and diffusely hypoechoogenic.

On the basis of these findings, the dog was admitted to MSUVTH, and enrofloxacin (5 mg/kg [2.3 mg/lb] IV, q 12 h) and lactated Ringer’s solution (100 mL/hr, IV) were administered to treat possible pyometra of the uterus. On the second day of hospitalization, because the dog had chronic anemia and variable thrombocytopenia, a bone marrow aspirate and core biopsy specimens were collected. Cytologic examination of the bone marrow aspirate revealed erythroid hyperplasia with adequate number of megakaryocytes but no evidence of neoplasia or infectious agents. Similarly, histologic examination of the core biopsy specimen (H&E stain) revealed erythroid and megakaryocyte hyperplasia. Bone marrow iron stores appeared adequate on the basis of visual estimation.

By the fourth day of hospitalization, the PCV had gradually decreased to 18%. This prompted the addition of doxycycline (5 mg/kg, PO, q 24 h) and prednisone (1.3 mg/kg [0.6 mg/lb], PO, q 12 h) to the treatment regimen to address possible tick-borne pathogens and immune-mediated erythrocyte destruction, respectively. Abdominal ultrasonography revealed that the uterine stump was gradually regressing in size. One day after the start of treatment with prednisone, the PCV increased from 18 to 23%, but the dog remained thrombocytopenic (76,000 platelets/µL). The dog’s appetite improved; because it had been afebrile for >36 hours, fluid administration was discontinued.

During this time, serum obtained from samples of the dog’s blood was submitted to the MSU Animal Health Diagnostic Laboratory for immunofluorescent antibody (IFA) testing for IgG antibodies against several tick-borne pathogens, including *Borrelia burgdorferi*, *Ehrlichia canis*, *E risticii*, *Rickettsia rickettsii*, and *Babesia canis*. The dog was seroreactive to *B canis* (IgG titer of 1:320) and *B burgdorferi* (IgG titer of 1:640) antigens. By use of the western blot technique, antibodies against several *B burgdorferi* protein bands were detected in the serum, but antibodies against *B burgdorferi* outer surface protein A (a marker of vaccination) were not detected. These results are consistent with natural exposure of the dog to *B burgdorferi*.

Samples of whole blood with EDTA and blood smears from the dog were sent to North Carolina State University College of Veterinary Medicine for light microscopic examination of blood smears and amplification of *Babesia* DNA by the polymerase chain reaction (PCR) assay. Small piroplasms consistent with *Babesia gibsoni* organisms (not *B canis*) were identified microscopically on blood smears stained with Romanowsky stain (Fig 1). Specific DNA amplification products of *B gibsoni*, but not of *B canis*, were detected in the blood by PCR assay.

Because the dog could have acquired the *Babesia* infection in Texas as a puppy, in Michigan at the breeding facility, or via the blood transfusion administered after ovariohysterectomy 3 weeks earlier, blood samples from the blood donor dog were submitted for serologic testing and PCR analysis. Results of IFA testing revealed that the donor dog was seroreactive to *B canis* (IgG titer of 1:10,000) antigens, but was seronegative for *B burgdorferi*, *E canis*, *E risticii*, and *R rickettsii* antibodies. *Babesia* organisms were not visible in erythrocytes on the donor’s blood smears; however, *B gibsoni* DNA, but not *B canis* DNA, was amplified from the donor’s blood samples via PCR technique. The PCR products detected in the 2 dogs were cloned and sequenced to confirm the PCR reaction specificity. The DNA sequences of the PCR products were identical to each other and also to known *B gibsoni* 18S rRNA genes (GenBank accession Nos. AF205636, AF271082, AF271081, AF175300, and AF75301; Fig 2).

After we were informed that the dog of this report was seropositive for *Babesia* and *Borrelia* antibodies, treatment with enrofloxacin was discontinued. The dose of doxycycline was increased to 5 mg/kg administered orally every 12 hours, for 30 days. The dog continued to receive prednisone to reduce the phagocytic activity of the reticuloendothelial system and thereby slow splenic RBC clearance. The dog was discharged in apparently stable condition pending delivery of imidocarb dibromopropionate for additional treatment of babesiosis.

At the time of the scheduled imidocarb injection 2 days later, the dog was weak, lethargic, and partially anorectic. Physical examination revealed dehydration, weight loss, and progressive splenomegaly. Results of serum biochemical analyses and a CBC indicated that the PCV had decreased to 15% without a concurrent reduction in total solids or platelet concentration; there...
was persistent hypoalbuminemia and hyperglobulinemia
with mild hyperbilirubinemia (bilirubin, 0.7
mg/dL; reference range, 0.1 to 0.6 mg/dL).
Ultrasonographically, enlargement of the uterine
stump was not detectable.

Imidocarb (6 mg/kg [2.7 mg/lb], IM) was adminis-
tered after treatment with atropine (0.02 mg/kg [0.01
mg/lb], IM). Two units of canine DEA 1.1-negative
packed RBCs were administered IV. After completion
of the transfusion, the PCV (20%) was lower than
expected. There was no evidence of an overt transfu-
sion reaction, but abdominal palpation revealed pro-
gressive splenic enlargement. The dose of prednisone
was increased slightly (1.6 mg/kg [0.7 mg/lb], PO, q 12
h) because of the apparent rapid splenic sequestration
of transfused RBCs. Within 2 days, the PCV had
increased to 30% and the platelet count was within ref-
ERENCE range; the dog was more active and its appetite
had improved. The dog was discharged to the owners,
and treatment with doxycycline and prednisone was to
be continued.

Two weeks later, the dog was returned to the MSU-
VTH for another imidocarb injection. The owners
reported that the dog was somewhat inactive but had
maintained a fair appetite. Abdominal palpation
revealed that the spleen had decreased in size. The
dog's PCV and platelet count had decreased (PCV, 23%;
platelets, 41,000/µL). The second dose of imidocarb
was administered IM after treatment with atropine as
described, and doxycycline administration was contin-
ued. During a period of 13 days, the dose of prednisone
was gradually reduced, and treatment was discontin-
ued. On day 45 after ovariohysterectomy, IFA testing
revealed IgG titers to
*B. burgdorferi*
and 1:320, respectively; these values were similar to
those detected initially.

On day 50 after ovariohysterectomy, the dog's
activity level and appetite were near normal, and
abdominal palpation revealed that the spleen had fur-
ther diminished in size. However, examination of
blood smears revealed persistent *B. gibsoni* parasitemia,
and results of a CBC indicated the dog was anemic
(PCV, 28%); and thrombocytopenic (61,000
platelets/µL). Therefore, a third dose of imidocarb was
administered IM (after treatment with atropine). At
this time, the doxycycline regimen was completed, and
treatment with metronidazole was begun (23 mg/kg
[10.5 mg/lb], PO, q 12 h).

After 7 days, the dog was re-evaluated at the MSU-
VTH. Despite further improvements in appetite, body
weight, and activity level, parasitemia and splenomegaly persisted, and the dog's hematologic
parameters were essentially unchanged. Metronidazole
treatment was discontinued, and administration of clindamycin (23 mg/kg/d, PO, administered in doses of
450 mg in the morning and 300 mg in the evening) was begun.

After 1 month of treatment with clindamycin, the
owners reported that the dog was behaving normally.
The dog had gained 17 lbs since the initial visit to
MSUVTH, and the spleen was normal in size on
abdominal palpation. The dog had mild anemia (PCV, 39%), platelet concentration within reference limits,
and mild neutropenia (3,140 mature neutrophils/µL;
reference range 3,230 to 10,850 neutrophils/µL).
*Babesia gibsoni* organisms were still visible in erythro-
cytes on microscopic examination of blood smears.
Neutropenia resolved with discontinuation of clind-
amycin treatment without concurrent reduction in
PCV or concentration of platelets. For a period > 11
months, the dog has remained clinically normal,
despite persistent parasitemia.

It is generally accepted that the mechanism of
transmission for *B. gibsoni* involves ixodid ticks as
intermediate hosts. However, there is speculation that
the parasite may also be transmitted transplacentally or
via direct blood contamination that could occur during
dog fights or the use of unclean, previously used equip-
ment such as needles or tail docking devices.

Controlled studies have yet to be performed to confirm
these modes of transmission. To the authors’ knowl-
edge, this is the first report of accidental transmission
of *B. gibsoni* via blood transfusion in a dog, although it
has been successfully transmitted experimentally to
dogs via transfusion of infected blood. It is possible
that the dog of this report was naturally infected prior
to administration of the blood transfusion and that
undergoing a surgical procedure resulted in clinical
signs of babesiosis. The *Babesia* status of dogs at the
breeding facility where the dog of this report had pre-
viously lived was not known. However, we received
information that the blood donor dog was a spayed
female American Pit Bull Terrier that had been found as
a stray in Michigan. The donor reportedly had several
scars suggestive of past involvement in dog fights but
otherwise appeared healthy. The breed of the donor
dog, its antibody titer to *Babesia* spp (detected by IFA
testing), and identification of *Babesia gibsoni* in the
donor's blood via PCR assay strongly suggested that
parasite transmission occurred at the time of the blood
transfusion. Among American Pit Bull Terriers tested in the southeastern United States, 55% were carriers of the parasite with infections that were mostly subclinical. Furthermore, development of signs of illness in the dog of this report coincided with the reported incubation period of *B. gibsoni* if the interval was calculated from the day of the blood transfusion.

A low index of suspicion for *B. gibsoni* infection, combined with the parasite's pleomorphic appearance, small size (approx 1 to 3 µm diameter), and variable parasitemia in hosts, makes *B. gibsoni* infection difficult to diagnose via microscopic examination of blood smears alone. These problems were encountered during the initial evaluation of the dog of this report. In addition, serologic cross-reactivity between *B. canis* and *B. gibsoni* can confound diagnosis when an IFA test for IgG antibodies is used. By means of PCR assay, species-specific regions of parasite DNA can be amplified, thus providing a way to definitively identify the infecting *Babesia* sp. Differentiation of the infecting *Babesia* sp has implications for prognosis and treatment. In the dog of this report, reliance solely on detection of a 4-fold increase in *B. canis* IgG antibody titer (via IFA testing) to confirm the diagnosis would have led us to the erroneous conclusion that the dog was not infected, largely because there was only a 2-fold increase between initial and subsequent titers. Possible explanations for the lack of a 4-fold or greater increase in IgG antibody titer may include suppression of the dog's immune response by corticosteroid administration or collection of the initial blood sample for titer determination too late in the course of the disease (ie, 17 days after infection).

The IFA and Western blot test results for *B. burgdorferi* indicated natural exposure of the dog of this report to that pathogen. The importance of that exposure to the dog's clinical problems was unknown; nevertheless, the clinical signs observed were inconsistent with borreliosis. In addition, the dog received a 30-day treatment with doxycycline, which is a recommended treatment for active *Borrelia* infection.

To the authors' knowledge, complete elimination of *B. gibsoni* from infected dogs has not been accomplished. Trimetrexate is the drug of choice to eliminate *B. canis* infection in dogs, but it is commonly ineffective for *B. gibsoni*, as was found in the dog of this report. Alternative drug treatments, such as diminazene aceturate or phenamidine isethionate, may resolve clinical signs of the disease and improve survival more effectively, but are not available in the United States. Doxycycline, metronidazole, and clindamycin have been reported to have some efficacy in treatment of babesiosis. *Babesia microti*, a species that can infect rodents and humans, has been treated with a combination of azithromycin and atovaquone. However, clinical trials evaluating the safety and efficacy of this drug combination for use in treating *B. gibsoni* infection in dogs have not yet been reported. Most dogs that survive the initial *B. gibsoni* parasitemia remain persistently infected carriers and may have relapses months to years after the initial episode. Long-term sequelae related to chronic immune stimulation, such as glomerulonephritis or polyarthritis, may develop.

In many cases of babesiosis, detection of immune-mediated hemolytic anemia, RBC autoagglutination, or the positive result of a direct Coombs' test may be misinterpreted as idiopathic immune-mediated hemolytic anemia. The administration of corticosteroids in dogs with babesiosis is controversial. In the acute stages of the disease, corticosteroids may reduce immune-mediated RBC destruction, but if used for long periods, these drugs may reduce splenic clearance of the parasite. The importance of splenic function is indicated by the fact that severe parasitemia is seen in splenectomized dogs infected with *B. gibsoni*. In the dog of this report, the PCV initially improved after beginning prednisone treatment despite a lack of evidence of peripheral immune-mediated RBC destruction (such as notable icterus, detection of spherocytes or autoagglutination, or positive result of a Coombs' test). This improvement, however, was not sustained, and the PCV decreased shortly thereafter. Treatment with prednisone was continued after detection of apparent splenic sequestration of transfused RBCs. On discontinuation of prednisone treatment, improvement in anemia was noted, which suggested that the drug may have been interfering with the dog's recovery. Although specific recommendations regarding the use of corticosteroids in dogs with babesiosis are not yet available, caution appears warranted in these cases.

The lack of severe bilirubinemia despite severe anemia is more typical of *B. gibsoni* infection (compared with *B. canis*) in dogs, although various degrees of hemolysis are seen with either *Babesia* sp. Hyperglobulinemia is commonly observed with both infections, reflecting antigenic stimulation from the parasites and the resultant RBC destruction. Thrombocytopenia is a consistent finding in *B. gibsoni*-infected dogs and may develop prior to detectable parasitemia and persist beyond resolution of anemia. Neutropenia, which was observed in the dog of this report during clindamycin treatment, may also be seen with *B. gibsoni* infection. Resolution of the neutropenia after clindamycin treatment was discontinued suggested that this could have been an adverse drug response. It was interesting that the dog of this report did not have leukocytosis on initial evaluation despite having a mild fever and parasite-induced extravascular RBC destruction. *Babesia canis* infection is rarely diagnosed in Michigan, and to the authors' knowledge, this is the first report of *B. gibsoni* infection in that state. Prior to 1979, *B. gibsoni* was thought to be limited to southern Asia, parts of Europe, North Africa, and the Middle East. However, *B. gibsoni* appears to be an emerging infectious disease of dogs in the United States. Dogs infected with *B. gibsoni* have been identified in eastern and southeastern states, and a distinct variant has been reported in California.

In evaluation of dogs with anemia, veterinarians should consider travel and housing history, as well as breed. Furthermore, blood donor dogs should undergo serologic, microscopic, and molecular screening for evidence of *Babesia* infection, regardless of the dogs' physical appearance, CBC results, or their geographic area of origin.
References