Concurrent bartonellosis and babesiosis in a dog with persistent thrombocytopenia

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- ➤ Bartonellosis can cause syncope or episodes of collapsing in dogs.
- ➤ In addition to hemolytic anemia, infection with *Babesia canis* can cause persistent thrombocytopenia.
- ➤ *Babesia canis* infection might not be detected by microscopic examination of a stained blood smear.
- ➤ A polymerase chain reaction assay can be used to diagnose babesiosis.

A 12-year old castrated male West Highland White Terrier weighing 7.2 kg (15.9 lb) was referred to our internal medicine service (day 1) for diagnostic evaluation of collapsing of approximately 1 year's duration; the frequency had increased during the previous month. Historically, some episodes were most consistent with syncope (short duration and rapid recovery with no obvious postictal phase), whereas urination and postictal signs of depression, indicative of a seizure, accompanied other episodes. The dog had a lifelong history of atopy that was accompanied by occasional bouts of severe pyoderma. One year earlier, after a seizure or syncopal episode, the referring veterinarian had detected marked increases in serum bile acids before and after eating, after which a laparoscopic liver biopsy and histologic examination revealed hepatic atrophy with hepatocellular vacuolation and multifocal lipid granulomas. Shortly before referral, prostatitis was diagnosed on the basis of a bloody preputial discharge and prostatomegaly, and antimicrobials were prescribed. A perianal gland adenoma was also diagnosed cytologically.

At the time of referral, physical examination abnormalities consisted of symmetrical prostatomegaly, perianal gland adenoma, gingival hyperplasia, and bilateral lenticular sclerosis. No changes were detected during a complete neurologic examination. Repeated cardiac auscultation failed to detect a heart murmur, and a normal sinus rhythm was evident on ECGs obtained during hospitalization. Hematologic abnormalities included poorly regenerative anemia (PCV, 26% [reference range, 33 to 58%]; slightly high reticulocyte concentration, 1.7% [reference range, 0 to 1.5%]), profound thrombocytopenia (18,000 platelets/µL [reference range, 181,000 to 350,000 platelets/µL]), lymphopenia (890 lymphocytes/µL [reference range, 1,000 to 5,000 cells/µL]), and mildly high serum alanine aminotransferase activity (113 U/L [reference range, 5 to 105 U/L]). The urine specific gravity was 1.035, and with the exception of 3+ bilirubinuria and trace proteinuria, results of urinalysis were unremarkable. Bacteriologic culture of urine failed to yield bacteria. Chronic intravascular hemolysis was suspected, but a Coombs' test did not detect erythrocyte-bound antibodies or complement. An aspiration biopsy of bone marrow was performed and yielded a highly cellular specimen with moderate megakaryocytic and erythroid hyperplasia with a regenerative shift in both cell lines. The myeloid-to-erythroid ratio was low (< 1). Myeloid numbers were adequate, and maturation was orderly. Mild erythroid dysplasia and rare erythrophagia were also detected. These findings were supportive of adequate platelet production and indicated that thrombocytopenia was a result of either platelet consumption or destruction. A coagulation panel consisting of measurement of prothrombin time, partial thromboplastin time, fibrinogen, and fibrinogen degradation products yielded results within reference ranges, indicating the absence of coagulation factor abnormalities or disseminated intravascular coagulopathy and thereby making platelet consumption a much less likely possibility. Pre- and postprandial serum bile acids values were again high (210 and 126 µmol/L, respectively [reference ranges, 0 to 20 mmol/L and 0 to 30 µmol/L, respectively).

Abdominal ultrasonography revealed slightly enlarged kidneys with mildly irregular surfaces accompanied by hyperechogenicity of the cortices and medulla. The urinary bladder wall was slightly thickened. There was a 1.9 × 1.2-cm hepatic cyst, small portal vessels, and enlarged hepatic arteries, indicating possible portal hypertension. The spleen was hyperechoic, and the right medial iliac lymph node was enlarged. The prostate was enlarged, irregular, and cavitated. Thoracic radiographs did not reveal abnormalities. A reciprocal antibody titer to *Bartonella vinsonii* (*berkhoffii)* antigens was 128 (reciprocal titers > 32 are considered indicative of prior exposure or active infection), whereas antibodies were not detected against *Babesia canis, Ehrlichia canis, Rickettsia rickettsii,* or *Borrelia burgdorferi* antigens. Use of a **polymerase chain reaction (PCR)** assay with EDTA-anticoagulated blood did not detect *Bartonella* DNA, possibly because of recent administration of antimicrobials. The collapsing episodes, poorly regenerative anemia, and presumptive immune-mediated thrombocytopenia were attributed to bartonellosis.

On day 2 of hospitalization, platelet concentration was 13,000 platelets/µL, and doxycycline (6.9 mg/kg

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[3.2 mg/lb], PO, q 12 h) administration was begun for treatment of bartonellosis. Prednisone (2.1 mg/kg [0.95 mg/lb], PO, q 12 h) was also administered in an effort to decrease or eliminate the presumptive immune-mediated platelet destruction and increase the dog's platelet concentration and thereby avoid life-threatening complications associated with myocardial or CNS hemorrhage.

Eleven days after initiation of treatment with doxycycline and prednisone, the dog's anemia had improved (PCV, 34%), and the platelet concentration had increased slightly to 45,000 platelets/µL. However, several liver enzyme activities were abnormal (alkaline phophatase, 789 U/L [reference range, 12 to 150 U/L]; alanine aminotransferase, 204 U/L; γ-glutamyl transferase, 18 U/L [reference range, 0 to 10 U/L]), most likely because of concurrent administration of prednisone. The *B vinsonii (berkhoffii)* reciprocal titer remained unchanged. Because of potentially better efficacy for treatment of bartonellosis, azithromycin was substituted for doxycycline and administered orally (8.7 mg/kg [3.95 mg/lb], PO, q 24 h for 1 week, then q 48 h for 6 weeks). Doxycycline administration was discontinued, and prednisone administration was continued at the same dosage.

The dog was examined approximately 1 month later (day 40), at which time the owner reported that the dog had extreme lethargy, hind limb weakness, and was unable to climb stairs. The dog weighed 8.2 kg (18.0 lb). The anemia had resolved (PCV, 40%), and although thrombocytopenia was still evident, platelet concentration had increased to 167,000 platelets/µL. The serum alkaline phophatase, alanine aminotransferase, and γ-glutamyl transferase activities remained increased (670, 166, and 26 U/L, respectively). Rectal scintigraphy confirmed shunting of hepatic blood flow (shunt fraction, 88.3% [reference value, < 15%]), and results of the scan were suggestive of a congenital portal-azygous hepatic shunt. Antibodies were not detected against *B vinsonii (berkhoffii)* antigens. Because the anemia had resolved and thrombocyte count was nearly within the reference range, castration was performed (without complications) to facilitate the medical management of the prostatitis. While general anesthesia was administered, a bone marrow aspiration biopsy was repeated. There was megakaryocytic hyperplasia with regenerative changes. Adequate numbers of all other cell lines were evident. Azithromycin administration was continued at 7.6 mg/kg (3.45 mg/lb), PO, every other day, and the prednisone dosage was decreased to 12.2 mg/kg (5.5 mg/lb), PO, every 48 hours, in an attempt to resolve the lethargy and muscle weakness that was presumptively associated with steroid-induced myopathy.

One month later (day 74), the dog weighed 9.0 kg (19.8 lb), and the owner reported improvement in attitude and overall activity. Muscle weakness in the hind limbs persisted but was less severe. The perianal tumor was notably reduced in size. Platelet concentration (297,000 platelets/µL) was within reference range, PCV was 36%, and mature neutrophilia (19,845 cells/µL) and monocytosis (4,410 cells/µL [reference range, 150 to 1,350 cells/µL]) were evident. Because the *Bartonella* infection appeared to have been successfully treated, azithromycin administration was discontinued. However, 6 weeks after discontinuation of administration of azithromycin (day 123), the dog returned for additional evaluation, because the owner reported polyuria, polydipsia, continued weakness in the hind limbs, and development of a pot-bellied appearance. Weight had increased to 10.3 kg (22.7 lb). These abnormalities were attributed to prednisone administration. The PCV (38%), neutrophil concentration (9,963 cells/µL), and serum alanine aminotransferase activity (69 U/L) remained within reference ranges; however, the dog was again thrombocytopenic (49,000 platelets/µL), and mild monocytosis persisted (1,476 monocytes/µL). The prostate was decreased in size and apparently nonpainful, and bacteriologic culture of urine again failed to yield bacteria. The reciprocal *B vinsonii (berkhoffii)* titer had decreased to 32. Persistent thrombocytopenia indicated continued immune-mediated platelet destruction; therefore, in addition to prednisone, azathioprine was prescribed at 2.4 mg/kg (1.1 mg/lb), PO, every 24 hours for 14 days and then every 48 hours for 1 month. The ultimate goal was to substitute azathioprine for prednisone to eliminate the undesirable adverse effects associated with corticosteroid administration.

On evaluation day 151, 2 weeks after stopping prednisone administration, the dog was again evaluated. Platelet concentration (188,000 platelets/mL), measured by the referring veterinarian prior to discontinuation of prednisone, was in the low normal range. Weakness and lethargy had resolved after discontinuation of prednisone administration, supporting a clinical diagnosis of corticosteroid-induced myopathy. However, the dog was again mildly anemic and severely thrombocytopenic (PCV, 33%; reticulocyte concentration, 0.5%; platelets, 18,000/µL). Liver enzyme activities remained within reference ranges. A reciprocal *B vinsonii (berkhoffii)* titer was again 32, further supporting successful therapeutic resolution of the *Bartonella* infection. During examination of a stained blood smear, large *Babesia* piroplasms were seen within erythrocytes (**Fig 1**). By use of a recently developed PCR assay that is capable of detecting as few as 50 parasites/mL of blood and facilitates differentiation of several *Babesia* spp, *B canis vogeli* DNA was amplified from blood samples[a] containing EDTA obtained on days 1, 40, and 151 of patient evaluation. The 340-base pair partial 18S rRNA gene amplicon obtained from the day-151 blood sample was cloned into a plasmid vector, and 3 clones were sequenced bidirectionally with an automated infrared DNA sequencer.[b] The sequences were found to have 99.99% sequence homology with the *B canis vogeli* sequences in Genbank (accession Nos. AY077719, AY072925, AF548006, AF547387, AJ009796, AY102162, and AY102163). These findings supported the possibility that babesiosis, as well as bartonellosis, was contributing to the intermittent, mild anemia and persistent thrombocytopenia in the dog. Two doses of imidocarb dipropionate[c] (5.0 mg/kg [2.3 mg/lb], IM) were given 2 weeks apart for treatment of babesiosis. Azathioprine administration was continued, as prescribed.

After treatment for babesiosis, anemia resolved, and platelet concentration steadily increased to refer-

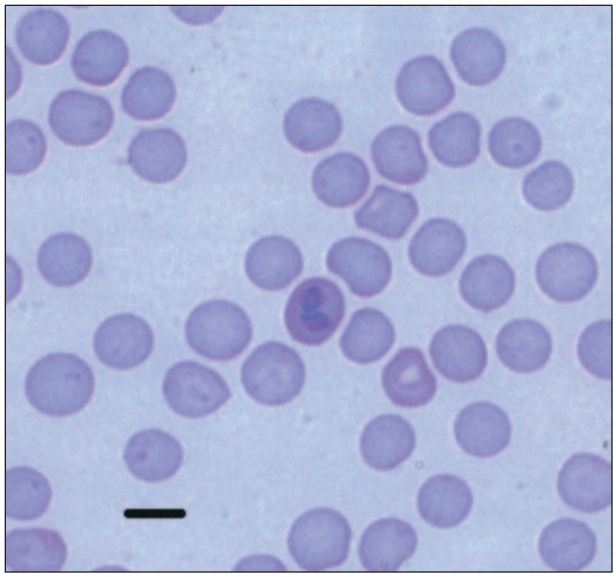

Figure 1—Photomicrograph of blood from a dog with bartonellosis and babesiosis. Notice the single erythrocyte containing 2 large piroplasms. Wright-Giemsa stain; bar = 8 µm.

ence range (day 165, 89,000 platelets/µL; day 205, 215,000 platelets/µL; day 237, 213,000 platelets/µL; day 373, 293,000 platelets/µL; day 601, 374,000 platelets/µL). The PCV and liver enzyme activities also remained within reference ranges. Two months after treatment, the owners reported substantial improvement in the dog's general health status (improved attitude and markedly increased activity level), and *Babesia* spp DNA was no longer detected in blood samples obtained on days 205, 237, and 373, by use of the PCR assay. Between days 165 and 601, antibodies were no longer detectable against *B vinsonii (berkhoffii)* and *B canis* antigens in 5 serum samples.

At the time of the diagnostic evaluation of this dog, our research group was attempting to characterize *B vinsonii (berkhoffii)* antibody kinetics after treatment with various antimicrobials. Therefore, several EDTA-anticoagulated blood and serum samples had been stored frozen during the initial period of evaluation and prior to finding *Babesia* organisms. After *Babesia* organisms were seen on day 151, retrospective as well as prospective *B canis* PCR assay and serologic testing was performed. Despite failure to see *B canis* organisms on Wright's-Giemsa-stained blood smears prior to day 151, *Babesia* DNA and low *B canis* antibody titers were detected retrospectively. Extensive retrospective screening of all blood smears and 2 bone marrow samples obtained prior to day 151 did not reveal intraerythrocytic organisms. After treatment with imidocarb dipropionate, *B canis* DNA could no longer be detected, reciprocal *B canis* antibody titers decreased from a reciprocal titer of 128 to nondiagnostic concentrations (reciprocal titers, < 64), and the PCV and platelet concentration remained within reference ranges. Upon retesting of the initial and 4 subsequent serum samples, variably low concentrations of *B canis vogeli* antibodies were detectable (reciprocal titers, 32 to 128). Presumably, the discrepancy between the initially reported negative *B canis vogeli* antibody titer and reciprocal titer of 128 obtained upon retesting of the same stored frozen serum sample indicated laboratory reporting error. To validate the *B canis* antibody titers, all samples were masked and tested in triplicate by the same technician during the same testing period with the same IFA antigen preparation. Identical IFA titers or only 1 dilution difference in titer was recorded for all samples, indicating minimal intra-laboratory variability.

This geriatric dog was evaluated because of a myriad of chronic health problems. Initially, the collapsing episodes, anemia, thrombocytopenia, and (potentially) high serum alanine aminotransferase activity were ascribed to *Bartonella* infection, which was diagnosed serologically. Endocarditis, myocarditis, and cardiac arrhythmias, at times accompanied by collapsing episodes, have been reported in dogs infected with *Bartonella* spp.[1] Regenerative hemolytic anemia or, less frequently, nonregenerative anemia and thrombocytopenia are hematologic abnormalities that have been reported in *Bartonella*-infected dogs.[1] Five months later, babesiosis was diagnosed and retrospectively determined to have been present for at least 5 months by use of PCR testing. The extent to which each organism, as well as other noninfectious age-related factors, contributed to the clinical and hematologic abnormalities at a specific point in time is impossible to definitively establish, particularly because the dog was treated with multiple antimicrobials and 2 immunosuppressive drugs during the period of clinical evaluation. However, after specific treatment for *B canis* infection was administered, there was rapid and sustained resolution of the abnormalities that persisted after administration of antimicrobials for bartonellosis. The PCR evidence and response to therapy strongly support a role for babesiosis as a cause of persistent thrombocytopenia in this dog. According to the owners, treatment of the *B canis* infection also induced a substantial additional improvement in the dog's general health status that had been poor for nearly 2 years. Although the duration of infection in this dog could not be established, both *B vinsonii (berkhoffii)* and *B canis* can cause chronic infections in dogs, which in many instances may be associated with minimal clinical abnormalities.[1]

Although thrombocytopenia has been reported in dogs infected with *Babesia* spp,[2-5] to our knowledge, babesiosis has not been implicated as a cause of persistent, unexplained thrombocytopenia in a clinical setting. Infections caused by *Babesia* spp have been classically diagnosed by viewing the organism on a Wright's-Giemsa-stained blood smear.[5-7] Because of the limited sensitivity of blood smear examination in chronically infected dogs, serologic testing is frequently recommended; however, although a controversial issue, the usefulness of serologic testing for confirmation of latent babesial infection has been recently questioned.[8] In our laboratory, reciprocal *B canis* antibody titers of 128 or greater are considered indicative of infection or prior exposure to *Babesia* organisms.[9,10] Although the occurrence of latent infection with *B canis* is well established in the clinical and research literature, the extent to which latently infected dogs develop chronic disease remains poorly documented,

as does the usefulness of serologic testing for confirmation of latent infection. In this dog, *B canis* antibodies were initially reported as undetectable, presumably as a result of a reporting error. Subsequent retrospective testing detected *B canis* antibody titers, most of which were less than the diagnostic cutoff value for the laboratory. Because the 2 initial reciprocal titers were 128, treatment with antimicrobials or corticosteroids may have contributed to decreased antibody production. Although seeing the organism has been the historically preferred method to establish a definitive diagnosis of canine babesiosis, the advent of PCR amplification of *Babesia* spp DNA can now be used to confirm the diagnosis when organisms are not seen and serologic testing detects nondiagnostic concentrations of antibodies, which is reported to be a frequent problem in young dogs infected with *B canis*.[6,7] The PCR assay used in this study was developed in our laboratory and is capable of detecting and differentiating several *Babesia* spp and subspecies known to exist in the United States.[a] In this dog, an amplicon was sequenced to confirm the validity of the PCR assay result.

This report illustrates the importance of considering co-infection with *B canis*, particularly in dogs that are infected with *E canis* or *B vinsonii* (*berkhoffii*).[11-13] All 3 organisms are known or thought (*B vinsonii*) to be transmitted by *Rhipicephalus sanguineus*, commonly referred to as the brown dog tick. In the southeastern United States, dogs that have positive results of indirect fluorescent antibody tests for *B vinsonii* (*berkhoffii*) antigens are frequently co-exposed or co-infected with *E canis* (36%) or *B canis* (57%).[13] Therefore, with additional research, the clinically relevant interactions among these 3 organisms may prove to be substantial. Because the dog in this report spent most of its life on a coastal sailing vessel, the owners had not observed ticks on the dog. However, the dog was boarded periodically in kennels in North Carolina and Florida and may have become infected there. Babesiosis should be considered as a differential diagnosis for dogs with other tick-transmitted infectious diseases that appear refractory to appropriate antimicrobial treatment. This is particularly relevant if an incomplete response is obtained with administration of doxycycline or azithromycin, which are antimicrobials that have limited efficacy for treatment of *B canis* infections.[14]

Neither culture nor PCR amplification of *B vinsonii* (*berkhoffii*) DNA from canine blood are sensitive diagnostic procedures, particularly if blood samples are obtained following administration of antimicrobials.[15,16] In the dog reported here, the initial and repeated *B vinsonii* (*berkhoffii*) reciprocal antibody titers were 128. After a protracted course of antimicrobials, the dog had no additional collapsing episodes, and *B vinsonii* (*berkhoffii*) antibodies were not detected in multiple serum samples obtained during a nearly 2-year follow-up period. Although the PCR assay failed to detect *Bartonella* DNA at the time of initial evaluation in our hospital, the dog had been treated with 2 antimicrobials for prostatitis immediately prior to referral. Prior antimicrobial treatment can reduce the template DNA in the blood sample to a concentration that is not detectable by use of PCR assay, without eliminating the *Bartonella* infection.

There is recent evidence that *Bartonella* infection can cause hepatic disease in human patients,[17-19] and *Bartonella* spp may contribute to hepatic damage in dogs. Infection with *B henselae* has been implicated as a cause of peliosis hepatis in a dog.[20] More recently, *B henselae* DNA and *B clarridgeae* DNA were amplified and sequenced from liver tissues of dogs with granulomatous hepatitis and hepatopathy of Doberman Pinschers, respectively.[21] In the dog in our report, treatment with doxycycline, followed by azithromycin, induced partial clinical improvement, resolution of anemia, and return of liver enzymes activities to reference ranges. It is also possible that these responses were related to suppression (but not elimination) of the babesia infection. Because improvement in several clinical, hematologic, and biochemical parameters occurred prior to administration of a specific antibabesial drug, it is also possible that *Bartonella* infection was contributing to the mildly increased liver enzyme activities in this dog.

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[b]LI-COR 4200 automated DNA sequencer, LI-Cor Biosciences, Lincoln, Neb.

[c]Imizol, Schering-Plough Animal Health Corp, Kenilworth, NJ.

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