Pathology in Practice

In cooperation with

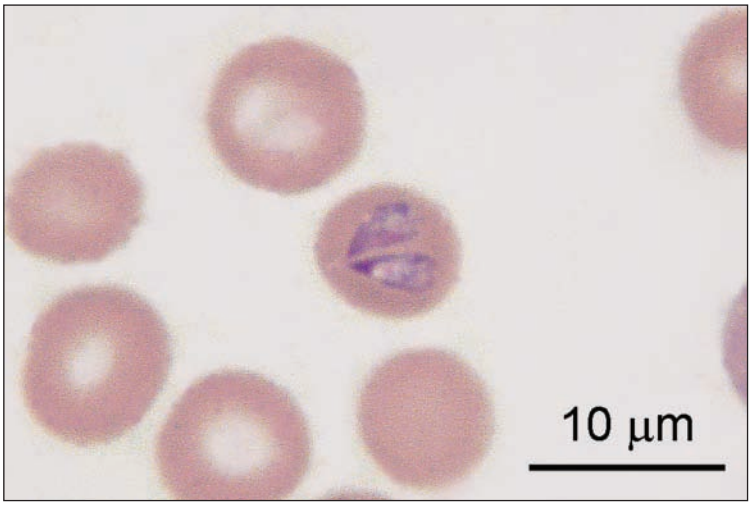

Figure 1—Photomicrograph of a peripheral blood smear from a dog that was evaluated for removal of a 3-cm-diameter raised pink dermal mass lateral to the base of the left ear; the dog had previously undergone splenectomy and was not currently receiving chemotherapy. Notice the presence of intraerythrocytic organisms. Bar = 10 µm.

History

An 11-year-old 20.8-kg (45.8-lb) castrated male Keeshond was evaluated prior to surgery to remove a 3-cm-diameter raised pink dermal mass lateral to the base of the left ear. The dog had an extensive medical history, including evaluation because of hypoalbuminemia in 2007. At that time, a splenic mass was identified via abdominal ultrasonography. A splenectomy was performed, and histologic examination of splenic tissue samples revealed multifocal nodular hyperplasia with hemorrhage. In 2008, a diagnosis of apocrine gland adenocarcinoma of both anal sacs with metastasis to an intraabdominal lymph node was made. Treatment consisted of a bilateral anal sacculectomy, removal of the enlarged abdominal lymph node, and chemotherapy. Chemotherapy started with administration of carboplatin but was changed to doxorubicin hydrochloride and finally to mitoxantrone because of progressive disease characterized by metastases to the lungs and medial iliac lymph nodes. Chemotherapy was discontinued owing to gastrointestinal adverse effects, and it had been 2 months since the patient had received the last dose of mitoxantrone.

Clinicopathologic and Cytologic Findings

Prior to surgery to remove the left ear mass, a CBC revealed moderate normocytic hypochromic regenerative anemia (RBC count, 3.18 × 10^6 RBCs/µL [reference interval, 5.7 × 10^6 RBCs/µL to 8.01 × 10^6 RBCs/µL]; hemoglobin concentration, 8.0 g/dL [reference interval, 13.8 to 20.3 g/dL]; Hct, 23.7% [reference interval, 39.2% to 55.9%]; PCV, 24% [reference interval, 39% to 58%]; mean corpuscular volume, 74.4 fL [reference interval, 64 to 75.2 fL]; mean corpuscular hemoglobin concentration, 33.7 g/dL [reference interval, 34.5 to 36.6 g/dL]; and absolute reticulocyte count, 105,576 reticulocytes/µL [reference interval, > 60,000 reticulocytes/µL]) with marked thrombocytopenia (27 × 10^3 platelets/µL; reference interval, 190 × 10^3 platelets/µL to 468 × 10^3 platelets/µL). The leukocyte count was considered normal (10.67 × 10^3 WBCs/µL; reference interval, 4.39 × 10^3 WBCs/µL to 11.61 × 10^3 WBCs/L) with a slight left shift (neutrophil count, 8.003 × 10^3 neutrophils/µL [reference interval, 2.841 × 10^3 neutrophils/µL to 9.112 × 10^3 neutrophils/µL]; band neutrophil count, 0.320 × 10^3 band neutrophils/µL [reference interval, 0 band neutrophils/µL]), and mild monocytosis (1.814 × 10^3 monocytes/µL; reference interval, 0.075 × 10^3 monocytes/µL to 0.85 × 10^3 monocytes/µL) was present. Serum biochemical analysis or urinalysis was not performed.

Microscopic examination of a blood smear revealed mild anisocytosis, slight poikilocytosis, and mild polychromasia. A low number of platelets with a moderate number of large platelets were noted. Red blood cells frequently contained 1 to 2 intracellular basophilic organisms. The organisms had a thick basophilic outer membrane with pale lavender to colorless internal structure. Within the RBCs, the organisms were centrally to eccentrically located and approximately 3 × 4 µm with an irregular or amoeboid to piriform shape (**Figure 1**).

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

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Additional Laboratory Findings

On the basis of the cytologic findings, infection with a *Babesia* sp was strongly suspected. Additional diagnostic tests were required to make a diagnosis beyond infection with a large *Babesia* sp. A PCR assay and DNA sequencing were performed as described elsewhere.[1] The DNA sequence was identical (nucleotide positions 1 to 845 of GenBank accession No. AY618928) to the described large *Babesia* sp identified in dogs that have been splenectomized or received chemotherapy. This organism is commonly referred to as *Babesia* sp (coco). The authors who originally characterized the DNA sequence of this *Babesia* sp believed that it was inappropriate to name this organism until the genotype of the > 100 named *Babesia* spp that have yet to be genotyped are known, thereby helping to minimize confusion regarding nomenclature names throughout the genus in the future.

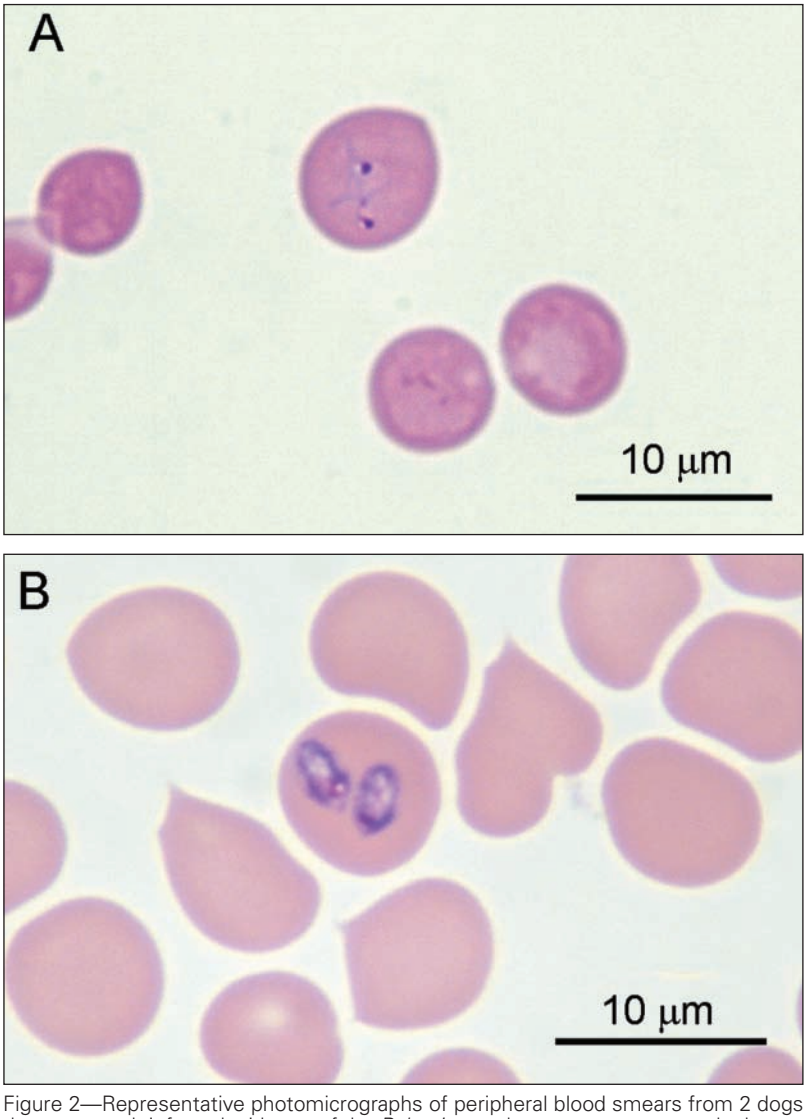

Figure 2—Representative photomicrographs of peripheral blood smears from 2 dogs that were each infected with one of the *Babesia* spp that are more commonly detected in dogs: *Babesia gibsoni* (a small *Babesia* sp; A) or *Babesia canis* (a large *Babesia* sp; B). In both images, bar = 10 µm.

Cytologic Diagnosis

Intraerythrocytic infection with organisms consistent with a large *Babesia* sp.

Comments

Babesiosis is a tick-transmitted hemoprotozoan parasitic disease that affects dogs, among other species, worldwide.[2] Sudden onset of lethargy and inappetence are the most common signs noticed by owners. In *Babesia*-infected dogs, common physical examination and laboratory diagnostic test findings include anemia, thrombocytopenia, pigmenturia, hyperglobulinemia, waxing and waning fever, and splenomegaly.[3] Historically, large and small species of *Babesia* have been identified in RBCs from dogs, ruminants, horses, and cats.[4] The large and small *Babesia* spp can sometimes be distinguished on cytologic evaluation (**Figure 2**). The small *Babesia* spp (eg, *Babesia gibsoni*) are typically between 0.5 and 2.5 µm in length and have a signet-ring appearance.[3] The large *Babesia* spp organisms (eg, *Babesia canis*) are typically between 3 and 5 µm in length, have a piriform shape, and exist singly or paired.[2,3]

Three large *Babesia* species or subspecies are known to cause clinical disease in dogs: *B canis vogeli*, *Babesia canis canis*, and *Babesia canis rossi*.[2] *Babesia canis vogeli* is a subspecies that is found in tropical and subtropical regions of most continents and is transmitted by the brown dog tick (*Rhipicephalus sanguineus*).[2] *Babesia canis vogeli* is also found in the United States and is the least pathogenic of the *B canis* subspecies.[2] *Babesia canis canis* is the subspecies found in Europe and parts of Asia and is transmitted by *Dermacentor reticulatus*.[2] *Babesia canis rossi* is the subspecies found in South America and is transmitted by the tick *Haemaphysalis elliptica*.[2] This is the most pathogenic strain of *B canis*.[2] Two additional large unnamed *Babesia* spp have been detected in clinically ill dogs. One organism was first detected in a Labrador Retriever from North Carolina that was undergoing chemotherapy for lymphoma.[5] The other large unnamed *Babesia* organism was identified in a Welsh Springer Spaniel from Britain.[6] These piroplasms are difficult to distinguish cytologically from the other 3 *B canis* species and subspecies. Several other dogs from the eastern United States infected with the large unnamed *Babesia* sp (coco) detected in the Labrador Retriever from North Carolina[5] have been identified.[1] Microscopic examination of stained blood smears cannot be used to accurately differentiate *Babesia* organisms to a species or genotype; therefore, species-specific PCR assays or DNA se-

quencing are necessary for the accurate identification of *Babesia* organisms.[1]

The origin of the large unnamed *Babesia* sp (coco) is unknown at this time, but it is presumed to undergo tickborne transmission. Clinical findings in dogs infected with the large unnamed *Babesia* sp (coco) are similar to those reported for dogs infected with other *Babesia* spp. Anemia and thrombocytopenia are the most common clinical findings, along with pigmenturia, hypoproteinemia, and high serum liver enzyme activities (alanine transaminase, aspartate transaminase, and alkaline phosphatase).[1]

Diagnosis of babesiosis depends on observation of *Babesia* organisms within infected erythrocytes, amplification of *Babesia* DNA extracted from blood or tissue samples via PCR assay, or positive results of serologic evaluation for antibodies against *Babesia* organisms.[2] Indirect fluorescent antibody tests are commonly used for detection of anti-*Babesia* antibodies, and titers ≥ 1:80 are supportive of exposure.[2] Among 7 dogs infected with the large unnamed *Babesia* sp (coco), most were initially identified as being infected via microscopic examination of blood smears, wherein large basophilic oval to piriform intraerythrocytic organisms were noted individually and in pairs.[1] On reviewing the historical data for those 7 infected dogs, dog fighting was not an apparent risk factor[1]; however, a history of tick infestation (4/7 dogs) and splenectomy (6/7 dogs) before diagnosis of *Babesia* infection appeared to be risk factors.[1] The Labrador Retriever[5] from North Carolina and the dog of the present report were both undergoing chemotherapy at the time of diagnosis of *Babesia* infection, and the dog of the present report also had been splenectomized.

The facts that 6 of the 7 dogs infected with the large unnamed *Babesia* sp (coco) had undergone splenectomy and 1 was undergoing chemotherapy[1] and that the dog in the present report had undergone splenectomy and was also receiving chemotherapy support the hypothesis that immunocompromised dogs are at risk for infection with the large unnamed *Babesia* sp (coco). It remains unknown whether domestic dogs are a primary reservoir host for *Babesia* sp (coco) and whether disease only develops when the immune system is no longer able to control the level of parasitemia.[1] On the other hand, it may be that there are a larger number of dogs exposed to this parasite, but only those with immune suppression or asplenia develop persistent infections and disease.[1]

Although no extensive studies on response to treatment in dogs infected with this *Babesia* sp (coco) have been performed to our knowledge, the following treatment protocols for dogs with *Babesia* infections have been used: imidocarb dipropionate (6.6 mg/kg [3.0 mg/lb], IM, twice [with 10 to 14 days between injections] after pretreatment with atropine [0.02 mg/kg [0.01 mg/lb], SC] 20 minutes prior to injection) or a combination of atovaquone (13.54 mg/kg [6.15 mg/lb], PO, q 8 h for 10 days) and azithromycin (10 mg/kg [4.55 mg/lb], PO, q 24 h for 10 days).[1] Dogs typically start to improve within 24 to 72 hours after beginning treatment, but for some patients, improvement may not be apparent for as long as 7 days.[2] Shortly after beginning treatment, microscopic examination of blood smears from infected dogs may reveal a marked reduction in numbers of detectable intraerythrocytic organisms; in some cases, no organisms are observed. Transfusions with packed RBCs may be necessary for dogs with severe anemia.[2]

Prevention is important to reduce the risk of infection with *Babesia* spp. Prevention can be accomplished by use of topical acaricides, prompt removal of ticks from pet dogs, thorough testing of blood donors, and control of interactions among dogs.[2] The case described in the present report emphasizes that immunocompromised dogs are at risk for development of infection with the large unnamed *Babesia* sp (coco) and the necessity of molecular diagnostic tests for the accurate identification of the *Babesia* sp in an infected dog; moreover, it is a reminder of the importance and value of performing microscopic examinations of blood smears from sick dogs.

For the dog of the present report, treatment with imidocarb dipropionate was initiated and surgery to remove the left ear mass was postponed. Once resolution of the *Babesia* infection was evident cytologically, the ear mass was removed. The dog continued to receive treatment for metastatic apocrine gland adenocarcinoma of both anal sacs, but neoplasia was progressive. The dog was euthanized because of neoplasia 9 months after the initial diagnosis of babesiosis.

References

1. Sikorski LE, Birkenheuer AJ, Holowaychuck MK, et al. Babesiosis caused by a large *Babesia* species in 7 immunocompromised dogs. *J Vet Intern Med* 2010;24:127–131.
2. Taboada J, Lobetti R. Babesiosis. In: Greene CE, ed. *Infectious diseases of the dog and cat*. 3rd ed. St Louis: Elsevier Inc, 2006;722–736.
3. Boozer AL, Macintire DK. Canine babesiosis. *Vet Clin North Am Small Anim Pract* 2003;33:885–904.
4. Allison RW, Meinkoth JH. Anemia caused by rickettsia, mycoplasma, and protozoa. In: Weiss DJ, Wardrop KJ, eds. *Schalm's veterinary hematology*. 6th ed. Ames, Iowa: Blackwell Publishing, 2010;199–209.
5. Birkenheuer AJ, Neel J, Ruslander D, et al. Detection and molecular characterization of a novel large *Babesia* species in a dog. *Vet Parasitol* 2004;124:151–160.
6. Holm LP, Kerr MG, Trees AJ, et al. Fatal babesiosis in an untraveled British dog. *Vet Rec* 2006;159:179–180.