Detection of a large unnamed *Babesia* piroplasm originally identified in dogs in North Carolina in a dog with no history of travel to that state

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**Case Description**—A 12-year-old 46-kg (101.2-lb) sexually intact male Labrador Retriever was evaluated because of lymphadenomegaly. The dog resided in Texas, and its travel history included many southeastern and eastern shore states but not North Carolina.

**Clinical Findings**—Following evaluation of the dog, a diagnosis of stage IVA intermediate- to large-cell lymphoma was made. A cyclophosphamide-hydroxydaunorubicin (doxorubicin)-vincristine-prednisone chemotherapy protocol was initiated. One week after the first chemotherapeutic treatment, a routine blood smear evaluation revealed single and paired intraerythrocytic large piroplasms that resembled *Babesia canis*. Via molecular testing, the organism was identified as a *Babesia* sp that had been detected previously in dogs in North Carolina.

**Treatment and Outcome**—The dog was administered imidocarb diproprionate (7 mg/kg [3.2 mg/lb], IM) on 2 occasions (3-week interval). At 1, 4, 15, and 50 weeks after the second treatment, blood samples were analyzed specifically for the North Carolina *Babesia* sp via PCR assay; the result of each assay was positive.

**Clinical Relevance**—Because of the morphologic similarity of the large piroplasm detected in dogs in North Carolina to *B. canis*, molecular testing of large piroplasms detected in dogs is needed to definitively identify the infective *Babesia* sp. In the dog of this report, the infection was not eliminated following treatment with imidocarb diproprionate, which may have been a result of the immunocompromised state of the dog or the drug’s ineffectiveness against this parasite. If imidocarb diproprionate is ineffective against the North Carolina *Babesia* sp, treated dogs may act as reservoirs of infection. (*J Am Vet Med Assoc* 2009;235:851–854)

A 12-year-old 46-kg (101.2-lb) sexually intact male Labrador Retriever was evaluated by the College of Veterinary Medicine Oncology Service at Texas A&M University because of lymphadenomegaly. Serum biochemical analyses revealed no marked abnormalities, but a CBC revealed mild neutrophilia (11,970 cells/μL; reference range, 3,000 to 11,500 cells/μL) and a large number of intermediate to large lymphoblastic lymphocytes in circulation. On the basis of clinical findings, a diagnosis of stage IVA intermediate- to large-cell lymphoma was made. A chemotherapy protocol involving cyclophosphamide, hydroxydaunorubicin (doxorubicin), vincristine sulfate, and prednisone was initiated. One week after the first chemotherapeutic treatment, a routine blood smear evaluation revealed the presence of single and paired intraerythrocytic large piroplasms (Figure 1). Examination of the Giemsa-stained blood smear revealed occasional parasitized erythrocytes (≤ 0.1% parasitemia) that contained piroform to irregularly shaped organisms similar in appearance to *Babesia canis* and that had colorless to pale blue cytoplasm and a red to purple nucleus. Piroplasms were most commonly seen in pairs, although single and intermediate forms were also evident.

The history of the dog was investigated further. The dog had been acquired as a puppy by the family and had resided in Texas since acquisition. The family and the dog had lived in the Houston area for approximately 1 month when the dog was a puppy, prior to moving to the College Station area. The dog traveled extensively with the family and had been to many of the southeastern and eastern shore states, including Louisiana, Alabama, Georgia, Tennessee, Virginia, and Pennsylvania. Ticks were found occasionally on the dog’s body, particularly during summer vacations to Louisiana; the most recent tick attachment was at least 6 months prior to the referral to the hospital. No information was available regarding the species of ticks located on the dog’s body.

The dog had never received a blood transfusion or been bitten by or had encounters with wild animals; however, it did sustain a few minor skin abrasions during an incident with a neighbors’ dog 5 years earlier (the neighbors’ dog had been euthanized in the interim for reasons unrelated to this incident).

Having detected the large piroplasmas, a venous blood sample was collected into a tube containing...
EDTA for a CBC. Results indicated that the PCV was 29% (reference range, 37% to 55%), and there was evidence of mild normocytic, normochromic anemia. The corrected reticulocyte percentage was 0.65%, indicating that the anemia was nonregenerative. Erythrocyte morphologic abnormalities included rare acanthocytes and schistocytes and mild polikilocytosis, which most likely resulted from erythrocyte fragmentation. Mild leukocytosis (25,600 cells/µL; reference range, 6,000 to 17,000 cell/µL) that was characterized by neutrophilia (22,528 cells/µL) and mild monocytes (2,048 cells/µL; reference range, 150 to 1,250 cells/µL) were detected. Platelet count was within reference range (239,000 platelets/µL; reference range, 200,000 to 500,000 platelets/µL). Serum biochemical analyses were not performed at this time.

Two days after the parasites were first observed in the examined blood smear, the dog was returned to the hospital for treatment. Another blood smear was prepared and examined microscopically; at this time, the number of parasitized cells was too low to determine parasitemia percentage (i.e., < 1 parasitized cell/100 fields at 50X magnification). The dog received 2 doses of imidocarb dipropionate (7 mg/kg [3.2 mg/lb]) administered IM into epaxial musculature (alternating body sides) at an interval of 3 weeks. The second dose is typically administered 10 to 14 days after the first dose but was delayed because of the dog’s poor tolerance of chemotherapeutic medications (neutropenia and vomiting). Prior to each imidocarb dipropionate injection, the dog was premedicated with glycopyrrolate (0.0043 mg/kg [0.002 mg/lb] and 0.0083 mg/kg [0.0038 mg/lb], SC, respectively) to minimize adverse treatment effects.

Six days following the first injection of imidocarb dipropionate, results of serum biochemical analyses indicated mildly high activities of alanine aminotransferase (140 U/L; reference range, 10 to 130 U/L) and alkaline phosphatase (606 U/L; reference range, 24 to 147 U/L); although high liver enzyme activities may develop in dogs with acute babesiosis, these abnormalities were most likely attributable to administration of prednisone that was part of the chemotherapy protocol. Serum biochemical analyses were not performed subsequently. However, a CBC performed 1 week following the second dose of imidocarb dipropionate revealed a mild improvement in the PCV (33%), and the leukocyte count (16,100 cells/µL) was within reference range. Mild thrombocytosis (694,000 platelets/µL) was also present and consistent with the concurrent administration of vincristine sulfate. At this time, examination of a Giemsa-stained blood smear revealed mild polikilocytosis but Babesia organisms were not detected.

Initially, a blood sample was obtained from the dog and tested for Babesia canis vogeli by use of a PCR procedure that involved primers that amplify the hemoproteozan 18S ribosomal RNA gene; this was followed by a nested PCR assay that was specific for detection of B canis vogeli and that involved species-specific primers (forward primer, 5'-GTGTTCCGAGTTTGCCATTC-3'; reverse primer, 5'-GAAAAGCCACAGTCCAAATA-3').

Despite the presence of B canis–like organisms in the blood sample, results of the initial PCR procedure were negative for B canis vogeli DNA. The primary PCR product was then used as a template in a nested PCR assay with primers 989A and 990A, which amplify an internal 18S rDNA fragment associated with most Babesia spp. An amplicon of the expected size (approx 1,000 base pairs) for piroplasms was obtained, cloned, and sequenced. A sequence similarity search (performed by use of a basic local alignment search tool) revealed that the obtained sequence was identical to the 18S rRNA gene for a large Babesia sp that had been first detected in a dog in North Carolina in 2004 (GenBank accession No. AY618928). Subsequent PCR tests that involved primers designed at the time specifically for this Babesia sp (forward primer, 5'-GAGTAGGACCGTAGCTC-3'; reverse primer, 5'-CCATGCAAACAAAGGATA-3') were performed 1, 4, 15, and 50 weeks after the dog received the second imidocarb dipropionate treatment. Results of each of these 4 assays were positive.

**Discussion**

Historically, 2 canine Babesia spp have been identified in the United States: the small piroplasm Babesia
gibsoni and the large piroplasm *B. canis vogeli*. Molecular characterization has recently revealed the presence of 2 additional *Babesia* spp that infect dogs in the United States. A small piroplasm (recently named *Babesia conradae*) has been detected in dogs in California; this piroplasm is morphologically similar to *B. gibsoni*. *B. conradae* is a large piroplasm (as yet unnamed) has also been detected; this piroplasm is morphologically similar to *B. canis vogeli*. The unnamed large piroplasm was first identified in a dog with lymphoma in North Carolina, and it was speculated that the immunosuppressed dog undergoing treatment for lymphoma might be an accidental host for this parasite. Since then, infections have been detected in other dogs, but to date, there have been no reports of this parasite in dogs in states other than North Carolina.

The dog of this report resided in Texas; it was being treated for lymphoma, as was the dog in North Carolina in which the unnamed large piroplasm was first identified. However, in the first case in North Carolina, the dog had clinical signs (eg, fever) and hematologic findings (anemia, leukopenia, and thrombocytopenia) that were consistent with babesiosis at the time of diagnosis. In the dog of this report, only slight nonregenerative anemia was evident, which was atypical given that regenerative anemia is typically associated with babesiosis. Furthermore, treatment of the dog in Texas was commenced 2 days after detection of the parasites during examination of a blood smear; at that time, blood smear examination was repeated and revealed fewer, rather than more, parasites than previously detected. Considering the immunocompromised state of the dog as a result of ongoing chemotherapy and immunosuppressive treatment and lack of antibabesial treatment, a reduction in the number of parasites was unexpected.

It was anticipated that administration of imidocarb dipropionate would successfully treat the parasitic infection because many large piroplasms, including *B. canis*, are susceptible to this drug. However, the dog of this report remained a carrier after treatment as determined via PCR testing for the parasite's DNA. Inasmuch as the dog had no infection-associated signs and was undergoing chemotherapy for lymphoma, it was decided that no other antibabesial treatment was warranted at that time. The failure to clear the infection via administration of imidocarb dipropionate may have been influenced by the immunocompromised state of the dog or may indicate that this parasite is not susceptible to that drug. Presently, there are no other approved drugs in the United States for treating dogs with babesiosis, although some success in treatment of dogs with *B. gibsoni* infections by experimental use of a combination of azithromycin and atovaquone has been reported.

To the authors' knowledge, efficacy of that drug combination in elimination of infections with large piroplasms such as *B. canis* or the North Carolina *Babesia* sp has not been documented to date.

Where and how the parasitic infection was acquired by the dog in Texas remain unknown. Although the dog had not traveled with its owners to North Carolina, it had been taken on trips to other states near North Carolina, where it is possible that the parasite is endemic but undetected at present. The possibility that the parasitic infection was acquired by the dog of this report during an altercation with a neighbors' dog that occurred 5 years previously cannot be ruled out. In the absence of reexposure, *B. canis* infections are eliminated after approximately 1 year, but other large piroplasms are known to persist for as long as 4 years (eg, *Babesia caballi* infection in horses) or for the lifetime of the animal (eg, *Babesia bigemina* infection in cattle). The pathogenicity of the North Carolina *Babesia* sp and associated immune response to infection in dogs are not well understood at present; therefore, it is not known whether the infection may persist for a period of 5 years. The blood-to-blood transfer of *Babesia* spp from one host to another is well documented and is thought to be a common mode of transmission for *B. gibsoni*, a small piroplasm of dogs.

Morphologically, the *Babesia* sp detected in smears prepared from blood samples collected from the dog of this report resembled *B. canis*. A PCR assay for *B. canis vogeli* DNA was carried out as a confirmatory test but unexpectedly failed to yield a positive result. Further molecular testing and gene sequencing identified the parasite as the unnamed *Babesia* sp that had been identified in dogs in North Carolina. It is possible that this parasite is more geographically widespread than is currently recognized because its morphologic similarity to *B. canis* may have resulted in inaccurate identification. In the past, definitive diagnosis of babesiosis (ie, *B. canis* infection) was made on the basis of blood smear examination because there was only 1 large piroplasm known to infect dogs in the United States. Thus, additional testing was not warranted, and it is possible that infections of dogs with this other large piroplasm have been misidentified as *B. canis* infections. Indeed, the dog of this report was thought to be infected with *B. canis vogeli* on the basis of the parasite morphology in blood smears, but this identification was not subsequently supported by results of PCR testing. Increased use of molecular diagnostic testing not only provides a more accurate means of identifying the infecting agent but also has the potential of revealing a wider geographic distribution of these parasites than is currently known.

**References**


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Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Effects of diet-induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses
Rebecca A. Carter et al

Objective—To determine the effects of diet-induced weight gain on glucose and insulin dynamics and plasma hormone and lipid concentrations in horses.

Animals—13 adult geldings.

Procedures—Horses were fed 200% of their digestible energy requirements for maintenance for 16 weeks to induce weight gain. Frequently sampled IV glucose tolerance tests were performed before and after weight gain to evaluate glucose and insulin dynamics. Adiposity (assessed via condition scoring, morphometric measurements, and subcutaneous fat depth) and plasma concentrations of insulin, glucose, nonesterified fatty acids, triglycerides, and leptin were measured on a weekly or biweekly basis.

Results—Mean ± SD body weight increased by 20% from 440 ± 44 kg to 526 ± 53 kg, and body condition score (scale, 1 to 9) increased from 6 ± 1 to 8 ± 1. Plasma glucose, triglyceride, and nonesterified fatty acid concentrations were similar before and after weight gain. Leptin and insulin concentrations increased with weight gain. Mean ± SD insulin sensitivity decreased by 71 ± 28%, accompanied by a 408 ± 201% increase in acute insulin response to glucose, which resulted in similar disposition index before and after weight gain.

Conclusions and Clinical Relevance—Diet-induced weight gain in horses occurred concurrently with decreased insulin sensitivity that was effectively compensated for by an increase in insulin secretory response. Obesity resulted in hyperinsulinemia and hyperleptinemia, compared with baseline values, but no changes in lipid concentrations were apparent. Preventing obesity is a potential strategy to help avoid insulin resistance, hyperinsulinemia, and hyperleptinemia in horses. (Am J Vet Res 2009;70:1250–1258)