Babesia gibsoni infection among dogs in the southeastern United States

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Objective—To identify subclinical Babesia gibsoni infection in American Pit Bull Terriers from the southeastern United States and to determine the genetic sequence of parasite DNA isolated from these dogs.

Design—Case series.

Animals—33 American Pit Bull Terriers and 87 dogs of various other breeds.

Procedure—Blood smears were examined for microscopic evidence of the parasite, and DNA was extracted from blood samples and used in a polymerase chain reaction (PCR) assay designed to amplify the small subunit ribosomal RNA gene sequence of B gibsoni. Amplification products of the expected size were sequenced, and sequences were compared with published sequences for B gibsoni isolates. Hematocrit, platelet count, mean platelet volume, WBC count, and eosinophil count were compared between dogs with positive PCR assay results and dogs with negative results.

Results—Results of the PCR assay were positive for 18 of the 33 (55%) American Pit Bull Terriers, including all 10 dogs with microscopic evidence of parasitemia. Only 1 of these dogs was clinically ill at the time blood samples were collected. Results of microscopic evaluation of blood smears and of the PCR assay were negative for the 87 other dogs. Hematocrit and platelet count were significantly lower in dogs with positive PCR assay results than in dogs with negative results.

Conclusions and Clinical Relevance—Results suggest that American Pit Bull Terriers in the southeastern United States may be subclinically infected with B gibsoni. However, subclinical infection was not identified in dogs of other breeds from the same geographic area. (J Am Vet Med Assoc 2002;220:326–329)

Babesia gibsoni is a small hemoprotozoan parasite reported to cause clinically important hemolytic anemia in dogs.14 The parasite was first identified in dogs and jackals from India in 191015 and is now considered endemic in northern Africa,16 the Middle East,6 and southern Asia.10,11 The first case of anemia in dogs.14 The parasite was first identified in the United States may be subclinically infected with B gibsoni. However, subclinical infection was not identified in dogs of other breeds from the same geographic area.10

B gibsoni was isolated from the blood of a dog that lived in Connecticut and had never traveled outside the United States.13 In 1991, 11 dogs raised in southern California reportedly developed severe hemolytic anemia and thrombocytopenia secondary to B gibsoni infection.15 Most recently, B gibsoni has been recovered from 9 dogs in North Carolina1 and 1 dog from Oklahoma.17 Between December 1999 and June 2000, clinicians at Auburn University’s Small Animal Clinic identified 6 American Pit Bull Terriers, including two 6-week-old littersmates, with severe hemolytic anemia associated with parasitemia with small Babesia organisms. This prompted the authors to test other dogs in this geographic area for evidence of subclinical infection with Babesia organisms.

Since the initial description in 1910, all dogs infected with small Babesia organisms were assumed to be infected with B gibsoni. Recently, however, Zahler et al13 described a small babesial parasite isolated from a dog in Germany that was genotypically distinct from B gibsoni organisms isolated from dogs in California and closely related to B microti, a babesial parasite that infects rodents and humans. Another recent study16 indicated that the DNA sequences of Babesia isolates from dogs in Oklahoma, North Carolina, Missouri, Indiana, and Alabama were identical to the sequences of isolates from dogs in Japan, Malaysia, and Sri Lanka but distinct from the sequence for the California strain.16 Almost all B gibsoni isolates recovered from dogs in the midwestern and eastern United States have been recovered from American Staffordshire Terriers and American Pit Bull Terriers.

The purposes of the study reported here were to identify subclinical B gibsoni infection in American Pit Bull Terriers from the southeastern United States and to determine the genetic sequence of parasite DNA isolated from these dogs. The 6 American Pit Bull Terriers identified to have severe hemolytic anemia associated with parasitemia with small Babesia organisms at Auburn University’s Small Animal Clinic between December 1999 and June 2000 were not included in the study.

Materials and Methods

Blood samples were collected from 20 American Pit Bull Terriers owned by a commercial breeder in Tuskegee, Ala, 11 American Pit Bull Terriers owned by a second breeder in Auburn, Ala who only sold dogs to private households, and 2 American Pit Bull Terriers owned by private individuals in Georgia and Alabama that were admitted to Auburn University’s Small Animal Clinic for orthopedic surgery. In addition, blood samples were collected from 37 American Foxhounds owned by a breeder in Georgia, 12 dogs of various breeds housed at the Lee County Humane Society...
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(Auburn, Ala), and 38 dogs of various breeds admitted to the Auburn University’s Small Animal Clinic that had blood taken for CBC during the same period blood samples were collected from the American Pit Bull Terriers.

Blood smears were created from all samples. For each dog, a 13-minute microscopic examination of 2 blood smears stained with Giemsa or Wright stain for B gibsoni organisms within RBC was performed. A CBC was performed on blood samples from the 33 American Pit Bull Terriers and the 38 dogs admitted to the Small Animal Clinic.

A polymerase chain reaction (PCR) assay was used to detect DNA from B gibsoni in blood samples. The DNA was isolated from blood samples with a commercial kit. Primers for the PCR reaction were based on the published sequence for the California B gibsoni isolate (GenBank accession No. L13729). The forward primer sequence was 5’-GATTAAGCCATGCGATGTCTAA-3’; the reverse primer sequence was 5’-CCATCATTCCAATTTCAAGGC-3’. The PCR reaction mixture contained 50 µl of master mix, 2 µl of magnesium chloride, 4 µl of primers (2 µl each for foward and reverse), 40 µl of water, and 4 µl of DNA template. The PCR reaction protocol consisted of an initial denaturation step of 5 minutes at 94°C followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 41°C for 30 seconds, and extension at 72°C for 45 seconds. A 7-minute extension at 72°C was performed at the end of the reaction. Amplification products were electrophoresed on 1.5% agarose gels. Results of the assay were considered positive if a distinct 441-bp band was seen. For samples with positive results, bands were extracted with a commercial kit, and amplified DNA products were sequenced with a commercial kit and automated sequencer.

To provide a positive control for the PCR assay, a cryopreserved sample of blood from a dog infected with the California strain of B gibsoni was obtained. Parasites used to infect this dog had been derived from the first dog with hemolytic anemia associated with infection with small Babesia organisms. Aliquots of the cryopreserved blood sample were inoculated (2 ml IV and 2 ml SC) into a splenectomized dog, as described. Blood samples were collected, and the PCR assay was performed as described.

Restriction enzyme assays were performed on amplified DNA from 10 of the American Pit Bull Terriers and the cryopreserved blood sample containing the California B gibsoni isolate. Briefly, 15 µl of amplified DNA isolated from gels was incubated with HaeIII at 37°C for 45 minutes. Cleaved DNA was electrophoresed on high-resolution agarose gels, and gels were stained with ethidium bromide and photographed.

Statistical analyses—Results of CBC (Hct, platelet count, mean platelet volume, WBC count, and eosinophil count) were compared between dogs for which results of the PCR assay were positive and dogs for which results were negative. Data were analyzed with the W statistic of Shapiro and Wilk to determine whether they were normally distributed. Data that were not normally distributed were tested for differences between groups with Student t-tests. Data that were not normally distributed were tested for differences between groups with the Wilcoxon rank sum test. Values of \( P < 0.05 \) were considered significant.

Results

Microscopic evaluation revealed parasites consistent with B gibsoni in blood samples from 10 of the 33 (30%) American Pit Bull Terriers (Fig 1). Parasites were pleomorphic and 1.0 to 2.5 µm in diameter and most commonly appeared as single ring forms in RBC. Only 1 of these dogs was clinically ill at the time parasitemia was detected. Four additional dogs had a history of acute hemolytic anemia that had responded to treatment with imidocarb (2 dogs) or to supportive care alone (2) several months previously. The other 5 dogs did not have any history of illness or any clinical signs of disease.

Results of the PCR assay were positive for 18 of the 33 (55%) American Pit Bull Terriers, including all 10 of the dogs with microscopic evidence of parasitemia. The 8 dogs with positive PCR assay results that did not have microscopic evidence of parasitemia were all clinically normal. Results of the PCR assay were positive for 12 of the 20 American Pit Bull Terriers owned by the commercial breeder in Tuskegee and 6 of the 11 American Pit Bull Terriers owned by the breeder who only sold to private households; results of the assay were negative for the 2 American Pit Bull Terriers that were family pets from single-dog households. Results of microscopic evaluation of blood smears and of the PCR assay were negative for all other dogs (37 American Foxhounds, 12 dogs from the humane society, and 38 dogs admitted to the Small Animal Clinic). Dogs for which results of the PCR assay were positive had a significantly lower Hct (mean, 31%; range, 16 to 45%), compared with dogs for which results were negative (mean, 41%; range, 30 to 52%). In addition, dogs for which results of the PCR assay were positive had a significantly lower mean platelet count (154,700/µl; range, 35,000 to 375,000/µl) and significantly higher mean platelet volume (11.2 fl; range, 9.4 to 13.1 fl) than dogs for which results were negative (256,900/µl [range, 105,000 to 554,000/µl] and 9.3 fl [range, 7.7 to 12.0 fl]). Total WBC and eosinophil counts were not significantly different between dogs for which results were positive and dogs for which results were negative.

For all samples with positive PCR assay results, amplified DNA was sequenced. Sequences were identical to each other but were not identical to published sequences for California B gibsoni isolates (GenBank accession No. L13729 and AF158709). Sequences did, however, match the small subunit ribosomal RNA gene sequences for B gibsoni isolates from Okinawa (Japan), Oklahoma, North Carolina, Indiana, and Missouri (GenBank accession No. AF271082, AF205636, and AF271081).
ic anemia, thrombocytopenia, lymphadenopathy, and splenomegaly. Dogs that survive the acute phase of infection may become chronic carriers and act as a potential reservoir for tick infection. Although anti-Babesia drugs have been shown to reduce the severity of disease in affected dogs, no drug has been proven effective in eliminating B gibsoni organisms from infected dogs. Results of the present study suggest that American Pit Bull Terriers in the southeastern United States may be subclinically infected with B gibsoni. However, subclinical infection was not identified in dogs of other breeds from the same geographic area.

Results of the present study further support the suggestion that multiple species of small Babesia organisms can infect dogs and that not all Babesia infections should be attributed to B gibsoni. Sequences of DNA amplified from blood samples from the present study matched sequences for isolates from Oklahoma, North Carolina, Indiana, Missouri, Japan, Malaysia, and Sri Lanka but were distinct from the sequence for the California isolate, which more closely resembles Thelieria spp. A third species of small Babesia organisms was isolated from a dog in Spain in 1998. This isolate closely resembles B microti, a parasite that primarily infects rodents but has been recognized as a zoonotic disease in humans in the eastern United States. New nomenclature likely will be required as phylogenetic testing reveals evidence of new species.

Dogs from the midwestern and eastern United States from which B gibsoni parasites have been isolated have almost exclusively been American Staffordshire Terriers and American Pit Bull Terriers, whereas the 11 dogs in California from which the organism has been isolated represented various breeds. The reasons for this predilection in American Staffordshire Terriers and American Pit Bull Terriers are unknown, and additional study is needed to determine whether American Staffordshire Terriers and American Pit Bull Terriers are genetically predisposed to infection with B gibsoni and to determine the modes of transmission.

Babesia organisms are transmitted by ticks, and known vectors outside the United States include the ixodid ticks Haemophysalis bispinosa and H longicorns. In the United States, Rhipicephalus sanguineus, the brown dog tick, is the suspected vector, but definitive transmission studies have not been done. All dogs included in the present study were examined for ticks at the time blood samples were collected, and none were found on any of the American Pit Bull Terriers. Ticks were found on the American Foxhounds, but none of these dogs were positive for Babesia infection. Breeders and owners of the American Pit Bull Terriers reported using aggressive tick control measures and did not believe that tick infestation was a problem in their dogs.

Transplacental transmission of parasites from dam to offspring may help explain why B gibsoni infection primarily involves American Staffordshire Terriers and American Pit Bull Terriers. However, although transplacental transmission of B gibsoni from bitch to offspring is likely to occur, it has not been document-

Discussion

Commonly reported clinical abnormalities in dogs infected with B gibsoni include fever, lethargy, hemolytic anemia, thrombocytopenia, lymphadenopathy, and splenomegaly. Dogs that survive the acute phase of infection may become chronic carriers and act as a potential reservoir for tick infection. Although anti-Babesia drugs have been shown to reduce the severity of disease in affected dogs, no drug has been proven effective in eliminating B gibsoni organisms from infected dogs. Results of the present study suggest that American Pit Bull Terriers in the southeastern United States may be subclinically infected with B gibsoni. However, subclinical infection was not identified in dogs of other breeds from the same geographic area.

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Figure 2—Gel electrophoretogram of amplification products obtained with a polymerase chain reaction assay that amplifies segments of the small subunit ribosomal RNA gene of Babesia gibsoni. Lane 1: molecular weight standards; 100 base pair (bp) increments. Lanes 2 and 3: products obtained following amplification of DNA in blood samples from 2 American Pit Bull Terriers in Alabama and cleavage of amplification products with HaeIII; notice the bands corresponding to products of 128, 135, and 178 bp (faint 390-bp bands in these lanes and in lanes 3 and 4 represent nonspecific amplification of canine genomic DNA). Lanes 4 and 5: products obtained following amplification of DNA in blood samples from 2 American Pit Bull Terriers in Alabama and cleavage of amplification products with HaeIII; notice the bands corresponding to products of 128, 135, and 178 bp. Lanes 6 and 7: products obtained following amplification of DNA in blood samples from 2 American Pit Bull Terriers in Alabama and cleavage of amplification products with HaeIII; notice the bands corresponding to products of 128, 135, and 178 bp (faint 390-bp bands). Lane 8: product obtained following amplification of DNA from a blood sample from a dog infected with the California B gibsoni isolate; notice the 461-bp band. Lane 7: product obtained following amplification of DNA from a blood sample from a dog infected with the California B gibsoni isolate and cleavage of amplification products with HaeIII; notice the 178- and 283-bp bands.

Restriction enzyme assays were performed on amplified DNA from the 10 American Pit Bull Terriers for which results of the PCR assay were positive and on amplified DNA from the dog inoculated with the California isolate. Amplification products from the American Pit Bull Terriers were cleaved at 2 sites with HaeIII, yielding 3 bands (128, 135, and 178 bp; Fig 2), whereas amplification products from the dog inoculated with the California isolate were cleaved at a single site, yielding only 2 bands (178 and 283 bp).

Discussion

Commonly reported clinical abnormalities in dogs infected with B gibsoni include fever, lethargy, hemolyti-
ed under controlled conditions. Two of the American Pit Bull Terriers in the present study were littermates for which results of the PCR assay were positive at 9 weeks of age; however, the puppies had been kept outside, and tick exposure could not be definitively ruled out. In our hospital, small Babesia organisms had been detected in two 6-week-old littermates, and in another report, B gisoni was found in blood smears from a dam and 3 puppies that were 3 days old. This latter report, in particular, strongly implies that the puppies were infected as a result of transplacental transmission, because the incubation period for B gisoni infection is 7 to 21 days.

Babesia parasites can also possibly be transmitted by direct blood contamination. Although dog fighting has been outlawed in the United States since 1976, certain enclaves for this illegal activity are likely still in existence, putting these dogs at risk for direct blood transmission. A previous report described B gisoni infection in a mixed-breed dog with acute hemolytic anemia that had been attacked by 3 pit bull-type dogs 2 months prior to examination. Although direct blood transmission of the organism could not be proven in that case, the time from exposure fits the incubation period for B gisoni.

Other methods of direct blood transmission that may occur in dog breeding operations include the sharing of instruments for surgical procedures such as ear cropping and tail docking and the reuse of needles for vaccinations. Breeders and owners of the dogs in the present study denied having used any of these practices and reportedly did not use the dogs for fighting. Commercial breeders regularly move dogs in and out of their kennels for breeding or whelping, often without any testing or quarantine procedures in place, and this may place the dogs at risk of infection. Some breeders of pit bull-type dogs reportedly ship dogs over-seas to countries where dog fighting is legal. One of these countries is Malaysia, an area in which B gisoni is endemic. Similarly, dogs belonging to US military personnel may be shipped to or from Okinawa, another area in which the parasite is endemic. In 1982, Farwell et al described 35 dogs infected with B gisoni and 11 dogs infected with B canis at Kadena Air Base in Okinawa. Most of these dogs were anorectic, lethargic, and anemic. Of 37 dogs tested, 31 had positive Coomb's test results. Although the dogs improved clinically following treatment with diminazene acetate or pentamidine isethionate, both drugs were ineffective in clearing the blood of B gisoni organisms. Thus, it seems likely that at least some of the approximately 500 dogs shipped annually from Okinawa to the United States by military personnel are carriers of the Okinawan strain of B gisoni, and the use of a PCR assay to screen dogs prior to shipment is recommended.

In the present study, median Hct and platelet count were significantly decreased, and mean platelet volume was significantly increased in dogs for which results of the PCR assay were positive, compared with dogs in which results were negative; however, changes were mild. Dogs that have these abnormalities and live in areas in which the organism is endemic may be carriers of Babesia spp, a PCR assay, as described in this report, could be used to identify these subclinically infected dogs. Diagnostic testing for PCR analysis is currently available through the North Carolina State University Tick-Borne Disease Testing Laboratory or through the Department of Pathobiology at Auburn University.

References


