Geographic distribution of babesiosis among dogs in the United States and association with dog bites: 150 cases (2000–2003)

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Objective—To identify the geographic distribution of babesiosis among dogs in the United States and determine, for dogs other than American Pit Bull Terriers (APBTs), whether infection was associated with a recent dog bite.

Design—Retrospective study.

Animals—150 dogs.

Procedure—Canine blood samples submitted to the North Carolina State University Vector-Borne Disease Diagnostic Laboratory between May 2000 and October 2003 for which results of a *Babesia*-specific polymerase chain reaction assay were positive were identified, and breed and geographic origin of dogs from which samples were obtained were recorded. History and hematologic abnormalities for dogs that were not APBTs were recorded, and possible associations with a recent dog bite were examined.

Results—Dogs positive for *Babesia* DNA were located in 29 states and 1 Canadian province (Ontario). *Babesia gibsoni* was the most commonly detected species, with *B gibsoni* DNA detected in blood samples from 131 of 144 (91%) dogs. Of the 131 dogs positive for *B gibsoni* DNA, 122 (93%) were APBTs. Of the 10 dogs positive for *Babesia canis vogeli* DNA, 6 were Greyhounds. In dogs other than APBTs, there was an association between having recently been bitten by another dog, particularly an APBT, and infection with *B gibsoni*.

Conclusions and Clinical Relevance—Results document an expansion of the known geographic range for babesiosis among dogs in the United States. Testing for babesiosis should be pursued in dogs with clinicopathologic abnormalities consistent with immune-mediated hemolytic anemia or thrombocytopenia, particularly if there is a history of a recent dog bite. (*J Am Vet Med Assoc* 2005;227:942–947)

Babesiosis is an emerging infectious disease among dogs in the United States.[1-5] However, there is little information about the epidemiology of the disease or the modes of transmission of the causative organisms. For instance, although babesiosis caused by *Babesia gibsoni* has most commonly been reported in dogs from the southeastern United States,[1,2] it has also been identified in dogs from Oklahoma[3] and Indiana,[4] suggesting that the condition may be more widespread than previously suspected.

Similarly, the 2 ticks—*Haemaphysalis longicornis* and *Haemaphysalis bispinosa*—known to be vectors for *B gibsoni* in other countries[6-8] have not been identified in the United States, with the result that the mode of transmission for *B gibsoni* in the United States has not been definitively determined. Although the brown dog tick, *Rhipicephalus sanguineus*, is frequently reported to be a competent vector for *B gibsoni*[9] and a few studies[10-12] have demonstrated the development of *B gibsoni* organisms in the midgut and salivary glands of *R sanguineus*, no definitive transmission studies under controlled conditions have been published. Results of the single study[13] claiming to have demonstrated that *R sanguineus* is a competent vector for *B gibsoni* have been questioned[7,14] because the researchers failed to use tick-proof kennels and some dogs unexpectedly developed *Babesia canis* infections. Given that puppies can develop *B gibsoni* infection as young as 2 weeks of age, perinatal transmission has been proposed as a possible route of transmission[1,15,16]; however, no controlled experiments have been performed to document perinatal transmission. Finally, because of the complexity of experimental tick transmission studies, inoculation of blood from a *B gibsoni*–infected dog into susceptible dogs remains the primary route of infection used in nearly all studies[7,17-20] involving experimental transmission of babesiosis.[9,19-22]

It has been speculated that blood transmission during dog fights could result in transmission of *B gibsoni*. Two breeds associated with dog fighting, the **American Pit Bull Terrier (APBT)** and the Tosa Inu, have been reported to have high rates of *B gibsoni* infection in Japan and Korea,[21-23] and most dogs identified in the United States with *B gibsoni* infection have been APBTs.[1-3,5] In APBT kennels with a history of *B gibsoni* infection, the prevalence of infection can be quite high. In a study[5] of 3 APBT kennels, for instance, 15% of the dogs were seroreactive to *B gibsoni* antigens and 48% of these seroreactive dogs had positive results when tested with a **polymerase chain reaction (PCR)** assay for *B gibsoni* DNA. In another study,[2] 55% of the dogs in a single APBT kennel had positive PCR assay results. In contrast, percentages of dogs housed in non-APBT kennels and animal shelters from similar geographic locations that had positive PCR assay results or were seroreactive to *B gibsoni* antigens were 0% and 1%, respectively.[2,5] Some *B gibsoni*–infected dogs in the

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United States that were not APBTs had a history of being bitten by or exposed to blood from an APBT. For instance, a mixed-breed dog from Indiana was reported to have been bitten by 3 APBTs prior to developing *B gibsoni* infection,[4] and a German Shepherd Dog from Michigan was identified as having *B gibsoni* infection shortly after receiving a blood transfusion from a *B gibsoni*–infected APBT.[24]

The purposes of the study reported here were to identify the geographic distribution of canine babesiosis in the United States and to determine, for dogs other than APBTs, whether infection was associated with a recent dog bite or tick attachment. Because 2 genetically distinct organisms have been identified as *B gibsoni*,[25] in the present study, the term *B gibsoni* is used to refer only to the Asian genotype (GenBank accession No. AF271081) and the California genotype (GenBank accession No. AF158702) is referred to as the western piroplasm.[26]

Criteria for Selection of Cases

Records of canine blood samples submitted to the North Carolina State University **Vector-Borne Disease Diagnostic Laboratory (VBDDL)** between May 2000 and October 2003 for testing with a *Babesia*-specific PCR assay[27] were reviewed, and dogs with positive PCR assay results were included in the study. Dogs for which multiple blood samples were submitted for testing during the study period were included if results for any sample were positive. Dogs for which multiple blood samples were submitted for testing during the study period were excluded if results for all samples were negative.

To increase the sample size for evaluation of possible associations between a recent dog bite or tick attachment and *B gibsoni* infection among dogs other than APBTs, dogs tested prior to May 2000 that were not APBTs and for which PCR assay results were positive were also included in the study. Data from these dogs were used only for evaluation of risk factors for *B gibsoni* infection among dogs other than APBTs.

Procedures

Breed and geographic location of dogs included in the study were recorded. Complete medical records were obtained from the submitting veterinary hospital for dogs included in the study that were not APBTs. Findings recorded for these dogs included age, breed, sex, geographic location, rectal temperature, PCV, nucleated RBC count, reticulocyte count, platelet count, and whether there was any history of a recent (ie, within the previous 6 months) dog bite or tick attachment. When information regarding history of a recent dog bite or tick attachment was not documented in the medical record, the owners were contacted by telephone to obtain pertinent information.

Statistical analyses—The χ^2 test was used to compare the proportions of dogs that were APBTs or Greyhounds between dogs with positive PCR assay results and dogs with negative PCR assay results. In addition, the χ^2 test was used to compare the proportions of dogs that were APBTs or Greyhounds between dogs that were tested for *Babesia* infection with the PCR assay and dogs that were tested for evidence of infection with other tick-borne pathogens during the same period. The Fisher exact test was used to compare proportions of dogs other than APBTs with a history of a recent (ie, within the past 6 months) dog bite, a history of a recent bite by an APBT, and a history of a recent documented tick attachment with proportions for a historical control group consisting of dogs infected with a different tick-transmitted pathogen, *Ehrlichia ewingii*.[28] Because multiple dogs from a single household were found to be infected with *B gibsoni*, analyses were repeated for households, rather than individual dogs. All analyses were performed with standard software.[a] For all analyses, values of $P < 0.05$ were considered significant.

Results

Seven hundred thirty-nine blood samples from 673 dogs were submitted to the VBDDL for testing with the *Babesia*-specific PCR assay during the study period, and results were positive for 144 of the 673 dogs. One hundred thirty-one of these 144 dogs tested positive for *B gibsoni* DNA, 10 tested positive for *Babesia canis vogeli* DNA, and 3 tested positive for *Babesia* DNA that did not match any currently described canine *Babesia* genotype. *Babesia canis canis*, *Babesia canis rossi*, and western piroplasm DNA were not detected in any of the blood samples, and none of the dogs tested positive for DNA from > 1 *Babesia* sp.

Of the 131 dogs positive for *B gibsoni* DNA, 122 (93%) were APBTs. Of the 10 dogs positive for *B canis vogeli* DNA, 6 were Greyhounds. Breed was not indicated for 19 canine blood samples submitted for *Babesia*-specific testing during the study period, but results for all 19 were negative. During the study period, 6,428 blood samples from 5,833 dogs were submitted to the VBDDL for testing for evidence of infection with other tick-borne pathogens, and breed was not reported for 754 of these dogs. All dogs for which breed was not reported were excluded from statistical analyses involving breed.

The proportion of APBTs positive for *Babesia* DNA (123/303 [41%]) was significantly ($P < 0.001$) higher than the proportion of dogs other than APBTs positive for *Babesia* DNA (21/351 [6%]). However, APBTs were significantly ($P < 0.001$) overrepresented among dogs that underwent *Babesia*-specific PCR testing (303/654 [46%]), compared with dogs that underwent testing for evidence of infection with other tick-borne pathogens during the same period (104/5,079 [2%]). The proportion of Greyhounds positive for *B canis vogeli* DNA (6/102 [6%]) was significantly ($P = 0.038$) higher than the proportion of dogs other than APBTs and Greyhounds that tested positive for *B canis vogeli* DNA (4/249 [2%]). However, Greyhounds were also significantly ($P < 0.001$) overrepresented among dogs that underwent *Babesia*-specific PCR testing (102/654 [16%]), compared with dogs that underwent testing for evidence of infection with other tick-borne pathogens during the same period (330/5,079 [6%]).

The 3 dogs positive for *Babesia* DNA that did not match any currently described canine *Babesia* genotype

had positive results when tested with a PCR assay designed to amplify DNA from most piroplasms but negative results when tested with PCR assays designed to amplify DNA from specific *Babesia* species and strains. These 3 dogs consisted of an APBT, a Labrador Retriever, and a Flat-Coated Retriever. The blood sample from the Labrador Retriever was subsequently subjected to further testing, including DNA sequencing, and a genotypically unique organism most closely related to *Babesia bigemina* was identified.[29] Blood samples from the other 2 dogs were not subjected to further testing.

Information on geographic location was available for 683 of the 739 blood samples submitted for *Babesia*-specific PCR testing during the study period. Dogs for which blood samples were submitted for *Babesia*-specific PCR testing were located in 37 states. Blood samples were not received from any dogs in the remaining 13 states for *Babesia*-specific PCR testing during the study period. Dogs positive for *Babesia* DNA were located in 29 states (**Figure 1**) and 1 Canadian province (Ontario). Results of *Babesia*-specific PCR testing were negative for all blood samples submitted from dogs in Alabama, Colorado, Hawaii, Minnesota, Mississippi, Rhode Island, Texas, and West Virginia. This included 11 dogs located in Texas; 7 located in Colorado; 6 located in Mississippi; and 1 each located in Alabama, Hawaii, Minnesota, Rhode Island, and West Virginia.

Nine of the dogs positive for *B gibsoni* DNA were not APBTs. In addition, 6 dogs tested prior to May 2000 that were not APBTs were found to be positive for *B gibsoni* DNA. Of these 15 dogs, 3 were mixed breeds; 3 were Boxers; 2 were Labrador Retrievers; and 1 each was a Rottweiler, an English Bulldog, a Border Collie, a Boston Terrier, a Belgian Malinois, a German Shepherd Dog, and a Cocker Spaniel. Five of the dogs were located in Georgia, 5 were located in Florida, 2 were located in North Carolina, 1 was located in Michigan, and 1 was located in Illinois. The remaining dog was a military working dog for which information on geographic location was not submitted. The dog from Michigan was a German Shepherd Dog that had received a blood transfusion from a *B gibsoni*–infected APBT prior to the diagnosis of immune-mediated hemolytic anemia; details for this dog have been published previously.[24] The 15 dogs ranged from 2 to 12 years old. Nine were female, and 6 were male.

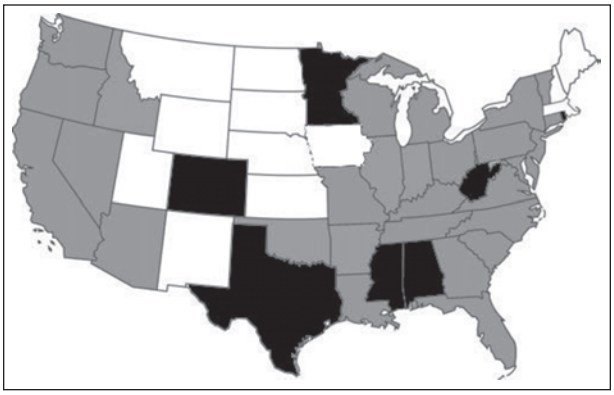

Figure 1—Geographic origin of canine blood samples submitted to the North Carolina State University Vector-Borne Disease Diagnostic Laboratory between May 2000 and October 2003 for which results of a polymerase chain reaction assay for *Babesia* DNA were positive (shaded grey) or negative (shaded black; Hawaii not shown). No samples from states in white were submitted for *Babesia*-specific polymerase chain reaction testing (Alaska not shown).

Medical records for 12 of the 15 dogs positive for *B gibsoni* DNA that were not APBTs were obtained from attending veterinarians. Rectal temperature at the time blood samples were collected for PCR testing was > 39.2°C (102.5°F) in 2 of 8 dogs. Packed cell volume was low (range, 15% to 27%; reference range, 33% to 58%) in all 11 dogs in which it was measured, and reticulocytosis (> 60,000 reticulocytes/µL) was detected in all 7 dogs in which reticulocytes were counted. Nucleated RBCs were seen during cytologic examination of blood smears from 11 of 12 dogs, with counts ranging from 2 to 16 nucleated RBCs/100 WBCs. Thrombocytopenia was detected in 8 of 9 dogs in which platelet count was measured, with platelet count ranging from 32 to 147 × 10^3/µL (reference range, 200 to 400 × 10^3/µL). Results of a Coombs' test were positive for 2 of 3 dogs that were tested.

Information on any history of a recent (ie, within the 6 months prior to diagnosis of babesiosis) dog bite or tick attachment was available for 12 of the 15 dogs positive for *B gibsoni* DNA that were not APBTs. Two of the remaining 3 dogs had been obtained as strays within 6 months prior to the diagnosis of babesiosis. The medical record of the third dog was not available for review, and the owner could not be contacted.

The proportion of *B gibsoni*–infected dogs that had a history of a recent dog bite (9/12) was significantly ($P = 0.014$) higher than the proportion of *E ewingii*–infected dogs (3/12) that had a history of a recent dog bite. All 9 of the *B gibsoni*–infected dogs with a history of recent dog bite were bitten by an APBT. Similarly, the proportion of *B gibsoni*–infected dogs that had a history of a recent bite by an APBT (9/12) was significantly ($P < 0.001$) higher than the proportion of *E ewingii*–infected dogs (0/12) that had a history of a recent bite by an APBT. The proportions of *B gibsoni*–infected dogs and *E ewingii*–infected dogs with a recent tick attachment (2/12) were identical.

Four of the *B gibsoni*–infected dogs that were not APBTs were housed on the same property as a *B gibsoni*–infected APBT; these 4 dogs represented 2 households. Two of the *E ewingii*–infected dogs lived in the same household. When households, rather than individual dogs, were considered in the analyses, the proportion of households with a *B gibsoni*–infected dog that had a history of a recent dog bite (7/10) was significantly ($P = 0.029$) higher than the proportion of households with an *E ewingii*–infected dog (2/11) with a history of a recent dog bite. Blood samples from 3 of the APBTs that had inflicted bite wounds were available for *Babesia*-specific PCR testing, and 2 of 3 were positive for *B gibsoni* DNA.

Discussion

Results of the present study suggest that canine babesiosis has a much wider geographic distribution in the United States than previously suspected. Previous

reports have identified dogs from Alabama,[2] Oklahoma,[3] Indiana,[4] North Carolina,[5] Michigan,[24] and Missouri[26] in which babesiosis caused by *B gibsoni* was diagnosed, and samples from dogs in Mississippi and West Virginia submitted to the VBDDL prior to May 2000 have been found to have *B gibsoni* DNA. In contrast, dogs for which results of *Babesia*-specific PCR testing were positive in the present study were from 29 states and Ontario. To our knowledge, babesiosis has not previously been diagnosed in 24 of these 29 states or in Canada. Because we did not evaluate travel histories for dogs in the present study, we cannot rule out the possibility that some of these dogs became infected when traveling to an area in which babesiosis has previously been identified. However, the wide geographic distribution of dogs positive for *Babesia* DNA makes it unlikely that this was the case for all infected dogs. The lack of cases in the central part of the United States may have been more a result of a lack of submissions from those states than an absence of canine babesiosis in this region.

Babesia gibsoni was the most commonly detected species in the present study, with *B gibsoni* DNA identified in 91% (131/144) of the samples with positive PCR assay results. This finding may represent a shift in the *Babesia* spp most commonly encountered in the United States. Prior to 1999, *B gibsoni* had been reported only rarely in the United States and *B canis vogeli* was the most commonly identified species.

Most of the dogs positive for *Babesia* DNA (123/144 [85%]) in the present study were APBTs. This high proportion of APBTs may have been attributable to submission bias in the sample population, an increased susceptibility to *B gibsoni* infection in the breed, or an increased risk of exposure to factors that enhance transmission among individuals of this breed. However, we believe that increased exposure to risk factors, rather than any genetic condition associated with increased susceptibility to *B gibsoni* infection, was responsible for the high proportion of infected dogs that were APBTs. Similarly, a high proportion of dogs positive for *B canis vogeli* DNA were Greyhounds (6/10). This likely was attributable to sample submission bias, as babesiosis has historically been recognized as being endemic in certain Greyhound kennels, particularly those located in the southeastern United States. The frequent transport of *Babesia*-infected racing Greyhounds in conjunction with failure to control *R sanguineus* infestations in kennel populations may have contributed to the maintenance of *B canis vogeli* infection among Greyhounds in the United States and Canada. The fairly recent practice of placing unsuccessful or retiring racing Greyhounds in adoptive homes may have contributed to the geographic dispersion of *B canis vogeli*–infected dogs in North America. Finally, the overrepresentation of APBTs and Greyhounds in samples submitted for *Babesia*-specific PCR testing, compared with samples submitted for testing for other tick-borne infections, was likely associated with an increased awareness of the possibility of babesiosis in these 2 breeds, as they are the most commonly reported breeds in previous studies[1,2,5,30,31] of babesiosis in the United States, and owners of these breeds frequently make independent requests to their veterinarians to have their dogs tested for babesiosis. All of this said, further studies are necessary to identify risk factors associated with *Babesia* infection in APBTs and Greyhounds.

Clinical and hematologic manifestations of *B gibsoni* infections among dogs other than APBTs in the present study were similar to those reported for infected APBTs and for other dogs naturally or experimentally infected with *B gibsoni*.[1,3,4,7,32] Hematologic abnormalities associated with *B gibsoni* infection are nearly identical to those associated with idiopathic immune-mediated hemolytic anemia and idiopathic immune-mediated thrombocytopenia. Thus, without specific testing, dogs with babesiosis can easily be misdiagnosed as having one of these conditions. It has been our experience that *Babesia*-infected dogs treated for prolonged periods with immunosuppressive drugs prior to initiation of anti-babesial treatment do not respond as well as dogs that are treated with anti-babesial drugs earlier in the course of the disease. Therefore, babesiosis should be considered in the differential diagnosis for dogs with clinicopathologic abnormalities, particularly anemia and thrombocytopenia, that could be consistent with immune-mediated hemolytic anemia or immune-mediated thrombocytopenia.

The clinical diagnosis of babesiosis can be challenging, as there is no clearly defined gold standard for the diagnosis of babesiosis in dogs. All of the currently available tests, including serologic testing for anti-*Babesia* antibodies, PCR assays for *Babesia* DNA, and microscopic examination of blood smears for the causative organisms, have limitations, and each can yield false-positive or false-negative results.[5,27,31,33-35] Microscopic examination of blood smears and the PCR assay have been shown to have a high level of agreement ($\kappa = 0.9$), with the PCR assay yielding more positive results.[5] Several studies[5,27,31,33-35] have shown that the sensitivity of serologic testing is < 100%; however, in many instances, anti-*Babesia* antibodies can be detected but results of microscopic examination, PCR testing, or protozoal culture are negative.[5,34,36] In fact, a previous study[34] identified so many dogs in which results of serologic testing were positive but results of protozoal culture were negative that the authors concluded that serologic testing was not a good indicator of latent *Babesia* infection. On the other hand, because true infection status was not known for the dogs that were tested, it could not be determined whether discrepancies between serologic and PCR testing or between serologic testing and protozoal culture were attributable to greater sensitivity or lower specificity of serologic testing. In a study[34] of dogs chronically infected with *B gibsoni* that were tested at 30-day intervals, the PCR assay was reported to have an overall sensitivity of 87%, with sensitivity increasing to 100% when results of 2 consecutive tests were interpreted in parallel. To our knowledge, no similar studies of the accuracy of testing for *B canis* infection have been performed. Because different *Babesia* spp result in different degrees of parasitemia during the acute and chronic phases of infection, it seems unlikely that sensitivity of a PCR assay will be the same for all species. It is also important to note that not all PCR assays use the same oligonucleotide primers for amplification, target gene,

method of amplicon detection, or controls, and these differences can result in as high as 200-fold differences in test sensitivity. Thus, clinicians must be cognizant of the technique and controls used by each diagnostic laboratory.[27] Because it is clear that there is no single perfect test for babesiosis in dogs, an integrated approach that combines multiple testing modalities should be used in dogs that are suspected of having babesiosis.

We have previously suggested that bite wounds and direct blood-to-blood transmission, as could occur during a dog fight, are possible routes for *B gibsoni* transmission. Of course, such potential routes of transmission are unlikely to ever be tested under controlled conditions. American Pit Bull Terriers are frequently aggressive toward other dogs, and this breed is the one most commonly associated with dog fighting in the United States. In our experience, owners of APBTs are often reluctant to report whether their dogs were bitten by another dog prior to developing babesiosis. Therefore, we did not believe that reliable epidemiologic results could be obtained regarding whether *B gibsoni*–infected APBTs had been bitten by another dog prior to developing babesiosis and chose not to pursue further investigation within that population of dogs. In dogs other than APBTs, there was an association between having recently been bitten by another dog, particularly an APBT, and infection with *B gibsoni*. The authors acknowledge that the historical control group—clinically ill dogs infected with *E ewingii*—used for statistical comparison was not ideal. However, because of the retrospective nature of this study, control dogs matched with case dogs on the basis of age, breed, sex, temporal occurrence, and geographic location were not available.

In conclusion, the present study documents an expansion of the known geographic range for babesiosis among dogs in the United States. We also detected a dramatic shift in the *Babesia* spp most commonly identified. *Babesia canis vogeli* was considered the most common cause of canine babesiosis in the United States, whereas in the present study, *B gibsoni* was identified more frequently during the study period and was widely distributed throughout the United States. In dogs other than APBTs, we found an association between *B gibsoni* infection and having been bitten recently by a dog, particularly an APBT. Clinicians should have a high index of suspicion for babesiosis in APBTs and Greyhounds with clinicopathologic abnormalities consistent with immune-mediated hemolytic anemia or immune-mediated thrombocytopenia. We recommend that clinicians ask clients whose dogs have these disorders whether their pets have recently been bitten by another dog, especially an APBT. Finally, we suggest that testing for babesiosis by means of microscopic examination of blood smears and serologic and PCR testing be actively pursued when a dog experiences an unexplained illness following a recent animal bite.

a. Epi-Info, version 3.3.2, CDC, Atlanta, Ga.

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