Detection of *Babesia gibsoni* and the canine small *Babesia ‘Spanish isolate’* in blood samples obtained from dogs confiscated from dogfighting operations

Todd J. Yeagley, JD, DVM; Mason V. Reichard, PhD; Julie E. Hempstead, DVM; Kelly E. Allen, MS; Lindsey M. Parsons, BS; Mellanie A. White, BS; Susan E. Little, DVM, PhD; James H. Meinkoth, DVM, PhD, DACVP

Objective—To determine the prevalence of *Babesia gibsoni* infection in dogs that were confiscated from dogfighting operations.

Design—Cross-sectional study.

Animals—157 pit bull–type dogs that were confiscated as part of dogfighting prosecution cases in Iowa, Michigan, Mississippi, Ohio, Pennsylvania, Virginia, and Washington and 218 randomly selected animal shelter dogs with no known history of dogfighting.

Procedures—Blood samples collected from confiscated dogs were tested for infection with *B gibsoni* by use of a nested PCR assay. Samples that yielded positive results underwent DNA sequencing to confirm infection with *B gibsoni*. Control blood samples collected from 218 randomly selected dogs in animal shelters (i.e., dogs that had no known involvement in dogfighting events) were also analyzed.

Results—Results of nested PCR assays indicated that 53 of 157 (33.8%) confiscated dogs were infected with *B gibsoni*.1 One (0.6%) dog was infected with the canine small *Babesia ‘Spanish isolate’* (also known as *Theileria annae*). To the authors’ knowledge, this is the first report of infection with this small *Babesia ‘Spanish isolate’* in a North American dog. Dogs with scars (indicative of fighting) on the face, head, and forelimbs were 5.5 times as likely to be infected with *B gibsoni* as were dogs without scars. Of the control dogs, 1 (0.5%) pit bull–type dog was infected with *B gibsoni*.

Conclusions and Clinical Relevance—Results indicated that *B gibsoni* is a common parasite of dogs confiscated from dogfighting operations and suggested that dogs with a history of fighting should be evaluated for infection with *B gibsoni*. *(J Am Vet Med Assoc 2009;235:535–539)*

Several species of *Babesia* are known to infect dogs. These protozoan parasites are characterized by pear-shaped intraerythrocytic inclusions called piroplasms; on the basis of size, these organisms can be divided into large (2.5 to 5.0 µm in length) and small (1.0 to 2.5 µm in diameter) *Babesia* spp. In the United States, the large *Babesia* spp that infect dogs are limited to *Babesia canis volgeli*1 and a *Babesia* sp that was recently isolated in North Carolina.2,3 Historically, small piroplasms of dogs were called *Babesia gibsoni* but have subsequently been genetically divided into at least 3 distinct albeit morphologically similar forms:4–6 *B gibsoni* (Asian genotype), *Babesia conradae*7 (formerly *B gibsoni* California genotype), and *Theileria annae*.7 *Babesia gibsoni* (Asian genotype) has been identified in dogs from Asia, North America, northern and eastern Africa, and Europe.8 *Babesia conradae* has only been detected in dogs from California,6 and *T annae* has only been detected in dogs from Europe. Genetic analyses9,10 have revealed that *T annae* is not closely related to other *Theileria* spp but is instead closely related to *Babesia microti*, the piroplasm that causes babesiosis in humans. Consequently, for the purposes of the present report, *T annae* will be referred to as the canine small *Babesia ‘Spanish isolate.’* In dogs, the disease processes associated with *B gibsoni* infection can range in severity from subclinical to fatal. In the acute phase, babesiosis can be associated with hemolytic anemia, thrombocytopenia, splenomegaly, lymphadenomegaly, anorexia, lethargy, pyrexia, and vomiting.10 Dogs often recover with treatment but remain subclinically infected with *B gibsoni*.11 *Babesia conradae* appears to be more virulent than *B gibsoni*.4,10 Infection with *B conradae* results in comparatively more severe parasitemia and more pronounced anemia. However, infection with *B gibsoni* in a mixed-breed dog that developed after a fight with a pit bull–type dogs was fatal.12 The canine small *Babesia ‘Spanish isolate’* is frequently associated with marked regenerative anemia, thrombocytopenia, and renal failure that results in azotemia and death.12–14
In the United States, *B. gibsoni* is considered an emerging infectious agent that is commonly detected in dogs that have a history of fighting with other dogs; most of the affected dogs identified to date were pit bull–type dogs or have fought with pit bull–type dogs. Infection with *B. gibsoni*, however, has been identified in Belgian Malinois, Border Collies, Boston Terriers, Boxers, Bulldogs, Cocker Spaniels, German Shepherd Dogs, Labrador Retrievers, Rottweilers, and mixed-breed dogs. Nevertheless, the prevalence of *B. gibsoni* infection has been found to be substantially greater among American Pit Bull Terriers.

Although *B. gibsoni* is transmitted by several species of ixodid ticks in certain areas of the world, a competent tick vector has not been definitively identified in the United States. Instead, transfer of infected *B. gibsoni* blood to native dogs during fights has been hypothesized to be at least partially responsible for transmission and maintenance of *B. gibsoni* infections in dogs. It has also been speculated that *B. gibsoni* infections are most common in pit bull–type dogs because these dogs are frequently used for fighting.

Pit bull–type dogs that have been bred, trained, or used for fighting often become part of the general dog population (eg, via adoption following dogfighting prosecution cases) where they have contact with other companion animals. In addition, dogs from fighting kennels that do not display adequate fighting behaviors (so-called gameness) may be sold to the public as pets. These dogs represent a likely source of *B. gibsoni* exposure to other companion canids. Thus, the purpose of the study reported here was to determine the prevalence of *B. gibsoni* infection among dogs confiscated from dogfighting operations.

**Materials and Methods**

**Identification of confiscated dogs**—A news alert service was used to locate dogs that were used as part of dogfighting prosecution cases. The animal shelters holding the confiscated dogs were contacted and asked to participate in the study. Participating animal shelters were provided with standard blood collection supplies and collection forms. For each dog, the identification number, color, sex, approximate age, and presence or absence of scars on the face, head, and forelimbs were recorded. Based on our understanding of dogfighters, we categorized the dog’s owner as follows: street dogfighter (person who owns 1 or 2 fighting dogs), hobbyist (person who owns <10 but >2 fighting dogs), or professional dogfighter (person who owns ≥10 fighting dogs). One blood sample (2 mL) from each dog was collected into an evacuated tube containing EDTA. All blood samples were shipped overnight to the Center for Veterinary Health Sciences at Oklahoma State University where they were tested for infection with *B. gibsoni* by use of a nested PCR within 2 weeks.

**Control dogs**—To provide controls for analysis, blood samples were also collected from dogs of any breed housed in the participating animal shelters that did not have a known history of dogfighting. Additional control blood samples were collected from similar dogs housed at the Stillwater Animal Welfare facility, Stillwater, Okla.

**PCR analysis of blood samples**—An aliquot (200 µL) of each whole blood sample underwent DNA extraction by use of a kit* according to the manufacturer’s instructions. A primary PCR assay was performed on a portion of the extracted DNA by use of primers that amplified the 18S rRNA gene for members of the order Piroplasmorida. A nested PCR assay was performed on a 1.0-µL volume of the primary PCR product from each sample by use of primers that amplified the 18S rRNA gene for all known *Babesia* spp in dogs. Blood samples from most of the confiscated dogs were also tested for infection with the tick-borne rickettsial agents (*Ehrlichia ewingii*, *Ehrlichia chaffensis*, *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Anaplasma platys*) by use of previously described PCR methods. To confirm that PCR inhibitors that would generate false-negative results were not present in the extracted DNA, all primary PCR samples that yielded negative results underwent an additional PCR amplification with primers that had high sequence homology with parasite and mammalian DNAs. To prevent cross-contamination, DNA extraction, PCR master-mix assembly, DNA amplifications, and DNA purifications were performed in separated and dedicated areas. Positive control procedures involved use of DNA extracted from blood containing a known piroplasm. Purified PCR water was used in negative control procedures.

To confirm that each dog with a positive PCR assay result was infected with *B. gibsoni*, the nested PCR product was sequenced by use of the reverse primer. The 18S rRNA genes of select *B. gibsoni*–positive samples were sequenced by use of overlapping forward and reverse primers at the Oklahoma State University Recombinant DNA/Protein Resource Facility. Basic local alignment search tool similarity searches were applied to individual consensus sequences from the 18S rRNA gene.

**Blood smears and assessment of parasitemia**—Upon arrival at the laboratory, 2 thin smears were made from each blood sample that was not hemolyzed and stained with a Romanowsky-type stain. Parasitemia in infected dogs was assessed as the number of piroplasms/1,000 RBCs observed microscopically (1,000× magnification) and expressed as a percentage.

**Statistical analysis**—χ² Tests were used to compare the prevalence of piroplasm infection among sex and age classes of confiscated dogs as well as among state of origin of the prosecution cases with which the dogs were associated. Odds ratios were calculated to determine the probability of a scarred dog being infected with a piroplasm, compared with dogs without obvious scarring. For all analyses, a value of *P* ≤ 0.05 was considered significant.

**Results**

**Confiscated dogs**—From March 2006 to November 2007, animal shelters housing confiscated dogs...
dogs from 19 dogfighting prosecution cases were contacted and asked to participate in the present study. Blood samples were obtained from 157 dogs in 9 (47.4%) separate dogfighting prosecution cases in Iowa, Michigan, Mississippi, Ohio, Pennsylvania, Virginia, and Washington. The confiscated dogs were all pit bull–type dogs. With regard to the dogfighter status of the dogs’ owners, 2 dogs were confiscated from persons categorized as street dogfighters, 22 dogs were confiscated from persons categorized as hobbyists, and 130 dogs were confiscated from persons categorized as professional dogfighters. Three dogs that were born following confiscation of 44 dogs owned by professional dogfighters in Mississippi were included in the study. The other dogs owned by professional dogfighters were from Michigan (n = 15), Ohio (34), and Virginia (37).

Fifty-four of the 157 (34.4%) dogs were infected with small piroplasms (Table 1). Of these infected dogs, none (0.0%) were owned by street dogfighters, 7 (4.5%) were owned by hobbyists, and 47 (29.9%) were owned by professional dogfighters. Results of DNA sequencing indicated that 53 (33.8%) of the dogs were infected with B gibsoni; 1 (0.6%) adult female dog that was confiscated in Mississippi was infected with the canine small Babesia ‘Spanish isolate.’ One of the dogs infected with B gibsoni was 1 of 3 puppies that were born after confiscation of the dam in Mississippi. Blood samples for PCR assay were collected from the dam and from the 3 puppies when they were 2 months old; test results for the dam and the other 2 puppies were negative. Of the 53 dogs that were infected with B gibsoni, only 1 dog developed clinical signs of babesiosis. The dog that was infected with the canine small Babesia ‘Spanish isolate’ did not have clinical signs of infection.

Almost the entire 18S rRNA gene of B gibsoni isolated from 1 of the dogs confiscated in Michigan and the 18S rRNA gene of the canine small Babesia ‘Spanish isolate’ were sequenced. Both sequences were submitted to GenBank (EU583386 and EU583387, respectively).

Among the confiscated dogs, those with visible scarring of the face, head, or forelimbs were 5.5 (95% confidence interval, 2.4 to 12.4) times as likely to be infected with piroplasms as dogs with no visible scarring. The prevalence of piroplasm infection in dogs with obvious signs of scarring (26.8%) was significantly (P < 0.001) higher than the prevalence in dogs with no visible scarring (3.7%) or in dogs for which the presence or absence of scarring was unknown (1.9%; Table 2). Significant differences in the prevalence of piroplasm infection among confiscated dogs were not detected with regard to the prosecution case (P = 0.346; data not shown), state in which the dogs were confiscated (P = 0.337), and sex (P = 0.345) or age (P = 0.099) of the dogs.

Most of the blood samples from confiscated dogs were hemolyzed upon arrival at the laboratory, which severely limited microscopic evaluation of blood smears. However, adequate smears were made from blood samples collected from 10 of the 157 pit bull–type dogs. Samples from all 10 dogs yielded positive nested PCR assay results, whereas only 2 of these samples had microscopically detectable parasitemias (< 0.1% and 0.5%).

**Control dogs**—Control blood samples were collected from 218 dogs of various breeds that did not have a known history of dogfighting. Of the 218 control blood samples, 18 were collected at 2 animal shelters that participated in the study and 200 were collected at the Stillwater Animal Welfare facility. One of the 218 (0.5%) control dogs was infected with B gibsoni. This control dog was a stray pit bull–type dog for which the history of fighting was unknown.

**Infection with tick-borne rickettsial agents**—Blood samples collected from 109 of the 157 (69.4%) confiscated dogs and from 3 of the 218 (1.4%) control dogs were also tested for infection with tick-borne rickettsial agents. Results indicated that none of the 112 dogs tested were infected with E ewingii, E chaffeensis, E canis, A phagocytophilum, or A platys.

### Table 1—Prevalence of infection with small piroplasms (Babesia spp) in pit bull–type dogs that were confiscated from dogfighting operations in the United States according to the geographic location of the dogs at the time of confiscation.

<table>
<thead>
<tr>
<th>State in which dogs were confiscated</th>
<th>No. of dogs confiscated (No. infected with small piroplasms*)</th>
<th>Prevalence of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>9 (4)</td>
<td>44.4</td>
</tr>
<tr>
<td>Michigan</td>
<td>15 (4)</td>
<td>26.7</td>
</tr>
<tr>
<td>Mississippi</td>
<td>47 (12)</td>
<td>25.5</td>
</tr>
<tr>
<td>Ohio</td>
<td>34 (12)</td>
<td>35.3</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>6 (3)</td>
<td>50.0</td>
</tr>
<tr>
<td>Virginia</td>
<td>39 (18)</td>
<td>46.1</td>
</tr>
<tr>
<td>Washington</td>
<td>7 (1)</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>157 (54)</td>
<td>34.4</td>
</tr>
</tbody>
</table>

*All infections involved Babesia gibsoni with the exception of infection that involved the canine small Babesia ‘Spanish isolate’ detected in 1 dog that was confiscated in Mississippi.*

### Table 2—Distribution of pit bull–type dogs that were confiscated from dogfighting operations in the United States according to age, sex, and evidence of scarring on the face, neck, and forelimbs.

<table>
<thead>
<tr>
<th>Scarring status</th>
<th>Male</th>
<th>Female</th>
<th>Unknown sex</th>
<th>Total No. of dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puppy</td>
<td>Adult</td>
<td>Puppy</td>
<td>Adult</td>
</tr>
<tr>
<td>Visible scarring</td>
<td>0 (0)</td>
<td>48 (21 [13.4])</td>
<td>1 (0)</td>
<td>34 (18 [11.5])</td>
</tr>
<tr>
<td>No visible scarring</td>
<td>3 (0)</td>
<td>25 (4 [2.5])</td>
<td>6 (1 [0.6])</td>
<td>29 (4 [2.5])</td>
</tr>
<tr>
<td>Unknown scarring</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (1 [0.6])</td>
<td>63 (22 [14.0])</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are the number of dogs with piroplasm (Babesia spp) infection and the prevalence of infection (%).*
Discussion

In the present study, at least 1 blood sample was collected from pit bull–type dogs owned by individuals in all 3 categories of dogfighters: street dogfighters, hobbyists, and professional dogfighters. Two dogs were confiscated in Virginia when a street dogfight was stopped by the police. To our knowledge, no other dogs were owned by the street dogfighters. Other samples were collected from dogs whose owners were considered hobbyists: 6 dogs were confiscated from a dogfighter in Pennsylvania, 9 dogs were confiscated from a dogfighter in Iowa, and 7 dogs were confiscated from a dogfighter in the state of Washington.

The remaining samples were collected from dogs that were owned by professional dogfighters. Fifteen dogs in Michigan, 34 dogs in Ohio, and 44 dogs in Mississippi were confiscated from professional dogfighters (3 additional dogs were born after confiscation of the dam in Mississippi). In addition, samples were obtained from 37 of 48 dogs that were confiscated as part of a prosecution case in Virginia; 1 of these dogs was euthanatized shortly after arriving at the shelter for health reasons, and samples could not be obtained from 10 other dogs.

Each group of dogs that were confiscated from hobbyists or professional dogfighters included a substantial number of infected dogs. In these 2 owner groups, the percentage of dogs that were positive for B gibsoni ranged from 23% to 50%. Fifty-three of the 155 (34.2%) dogs confiscated from hobbyists or professional dogfighters were positive for B gibsoni. One of the 157 (0.6%) dogs was positive for the canine small Babesia ‘Spanish isolate’ (ie, T annae). To our knowledge, this is the first report of this organism in a canid in North America.

The prevalence of B gibsoni infection is significantly higher in pit bull–type dogs than it is in the general domestic dog population.15 In the present study, 3 blood samples were obtained from dogs of various breeds at the Mississippi shelter and 15 blood samples were obtained from dogs of various breeds at the Ohio shelter for use as control samples. None of those dogs had a history of fighting, and analysis revealed that none were infected with B gibsoni. Similarly, 200 dogs without a known history of fighting from the Stillwater Animal Welfare facility were tested for infection with B gibsoni; results were positive for 1 dog, which, interestingly, was a pit bull–type dog. Although the Stillwater facility was not one of the shelters that provided blood samples collected from confiscated dogs, the test results for the Stillwater control samples are still relevant because B gibsoni has been present in Oklahoma for over a decade16 and because pit bull–type dogs have been previously confiscated as part of dogfighting prosecution cases in Oklahoma.

The transmission of B gibsoni is thought to occur primarily through the transfer of infected blood during a dogfight. This theory, coupled with the use of the pit bull–type dogs as the predominant fighting dog, serves to explain the high prevalence of B gibsoni infection among pit bull–type dogs, compared with findings in other breeds. Our observation of dogfight videos supplied by the Humane Society of the United States did not reveal that the dogfights are particularly bloody. However, on the basis of the video data and from speaking with dogfighting experts, lacerations of the oral cavity frequently occur during these fights. Commonly, a dog may also bite through its own lip during a fight (a so-called fanged dog), which would release a sufficient volume of blood to transmit B gibsoni.

In the present study, confiscated dogs with scars on the face, head, and forelimbs were 5.5 times as likely to be infected with B gibsoni as confiscated dogs without obvious scarring. This finding supports the contention that B gibsoni is transmitted via transfer of infected blood during a fight. The high prevalence of B gibsoni infection among pit bull–type dogs associated with dogfighting combined with their frequent interstate transportation likely contributes to the maintenance and spread of the protozoan parasite among pit bull–type dogs.

The fact that a small number of infected dogs in the present study lacked scarring suggests that there may also be another route of transmission. Although iatrogenic transmission of B gibsoni may be possible, it is not a likely route of transmission. Iatrogenic transmission would result in a prevalence of infection that was independent of scarring status, which was not confirmed by the results of our study. Instead, the data strongly suggested an association between scarring and infection. In addition, anecdotal reports indicate that professional dogfighters are aware that B gibsoni is blood borne and practice sterile technique to avoid iatrogenic transmission. This is also supported by the data obtained in the present study. Twelve of the 34 (35.3%) dogs that were confiscated from a professional dogfighter in Ohio were infected with B gibsoni. Total removal of the pinnae (a common practice of dogfighters) had been performed in 4 of the 34 Ohio dogs, but none of those dogs were infected with B gibsoni.

Transplacental transmission has also been referenced anecdotally as another source of B gibsoni infection in dogs.16 In the present study, the data regarding this method of transmission were equivocal. Ten puppies were included in the study population, only one of which was infected with B gibsoni. That particular puppy was born in a foster home along with 2 other littermates after confiscation of its dam. Blood samples for PCR assay were collected from the dam and from the 3 puppies when they were 2 months old; test results for the dam and 2 puppies were negative. The B gibsoni–positive puppy could have been infected transplacentally if the dam was infected at a level less than the PCR assay’s limit of detection or if the dam’s infection had been eliminated after the parasite had been transmitted to the puppy. Alternatively, the puppy could have been infected after birth. Thus, the data did not provide any support for transplacental transmission of B gibsoni.

Although B gibsoni is transmitted by ixodid ticks in other parts of the world,17,18 acarines do not seem to be a factor in the transmission of B gibsoni in North America. Blood samples obtained from 3 control dogs and 109 confiscated pit bull–type dogs included in the present study were also tested by use of PCR assays for infection with tick-borne rickettsial agents (E chaffeensis, E chafensis E canis, A phagocytophilum, and A phagocytophilum). Of the 109 confiscated dogs that were tested for infection with tick-borne rickettsial agents, 32 were infected.
with *B. gibsoni* and 1 was infected with the canine small *Babesia* ‘Spanish isolate.’ However, test results indicated that none of the 109 confiscated dogs were infected with any of the tick-borne rickettsial agents investigated, which suggests that *B. gibsoni* was transmitted via a method other than a tick bite.

While residing at the respective shelters, all of the confiscated pit bull–type dogs in the present study were examined by a veterinarian and were observed for at least 2 weeks before being euthanatized or moved to other shelters. This observation period allowed for the development and detection of clinical signs associated with babesiosis. Of the 53 dogs that were infected with *B. gibsoni*, only 1 dog developed clinical signs of babesiosis. The 1 dog that was infected with the canine small *Babesia* ‘Spanish isolate’ did not have clinical signs of infection.

Detection of the canine small *Babesia* ‘Spanish isolate’ in the confiscated pit bull–type dog is noteworthy because it indicates another means by which nonnative parasites and foreign animal infectious diseases may be brought into the United States. It is our understanding that professional dogfighters are importing and exporting dogs from Europe and other countries. Additionally, *B. gibsoni* has been recently identified in pit bull–type dogs from western Europe and South Africa.

References