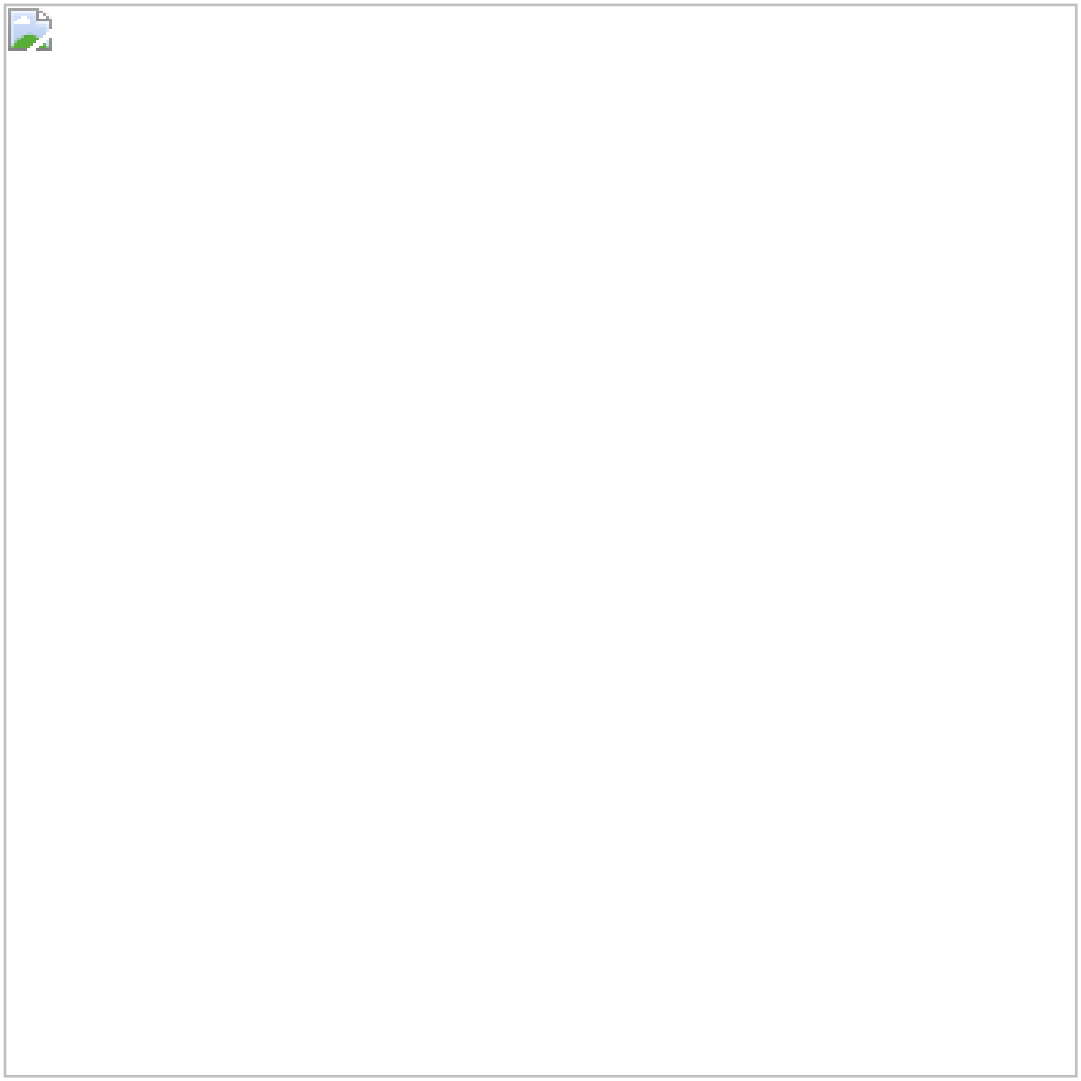

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Experimental *Babesia gibsoni* Infection in Coyotes (*Canis latrans*)

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ABSTRACT: Four 5 mo old captive raised coyotes (*Canis latrans*) were experimentally inoculated with approximately 1×10^6 *Babesia gibsoni* organisms. Parasites were detected 1 wk post-inoculation in all coyotes with maximum parasitemia of 8–11% occurring at 3–4 wk. Parasitemias remained at or above 1% for at least 12 wk and were still detectable 20 wk post-inoculation. All experimentally infected coyotes developed pale mucous membranes, splenomegaly, and a positive heme reaction in urine while one coyote exhibited mild depression and inappetence. Infected coyotes also developed a regenerative anemia, thrombocytopenia, and neutropenia. The mild clinical signs coupled with the high level and long duration of parasitemia indicate that coyotes could serve as reservoirs for *B. gibsoni.* Entrance of this foreign parasite into the United States suggests the need for strict quarantines and thorough health and blood film examinations for imported animals.

Key words: *Babesia gibsoni,* babesiosis, *Canis latrans,* coyote, hemoprotozoan.

Babesia species are tick-transmitted protozoan blood parasites that infect numerous vertebrates worldwide. Infections are characterized by a regenerative hemolytic anemia, anorexia, depression, multi-organ dysfunction, and occasionally death. Throughout the world, canine babesiosis occurs as the result of infection with either the "large" *Babesia, B. canis,* or a "small" *Babesia, B. gibsoni,* but in the United States cases have primarily been attributed to *B. canis.* With one exception (Anderson et al., 1979), *B. gibsoni* was not reported in North America until the 1990s when there were reports from California (Conrad et al., 1991; Yamane et al., 1994; Wozniak et al., 1997) and various midwestern and eastern states (Birkenheuer et al., 1999; Irizarry-Rovira et al., 2001; Kocan et al., 2001; Macintire et al., 2002). Recently, nucleotide sequence comparisons of the entire 18S nuclear subunit ribosomal RNA gene of all known isolates of *B. gibsoni* revealed there are three genetically distinct small canine *Babesia* parasites, two of which occur in the United States (Kjemtrup et al., 2000; Zahler et al., 2000a, b). In North America, *B. gibsoni* sensu stricto has been found predominantly in American Staffordshire terriers (AST) and seems to be maintained within their breeding colonies through mechanical blood transfer and possibly vertical transmission (Abu et al., 1973; Correa, 1974; Irizarry-Rovira et al., 2001). It is further speculated that the sudden increase of *B. gibsoni* cases in the United States has been the result of importation of infected dogs from endemic locations outside of North America such as Okinawa, Japan (Farwell et al., 1982). Currently, there are no drugs available that are effective in clearing the parasite from infected dogs.

Domestic dogs chronically infected with *B. gibsoni* not only increase the potential risk of spreading the disease among themselves but may also pose a risk to wild canids where the impact of infection is unknown. Although naturally occurring *B. gibsoni* infections in wild canids have not been reported in the United States, it is known that interspecies interactions of domestic and wild canids occur through both natural and man-made conditions (Mengel, 1971; Freeman and Shaw, 1979; Schmitz and Kolenosky, 1985). The purpose of the present study was to evaluate susceptibility of coyotes (*Canis latrans*) to experimental *B. gibsoni* infection and to assess the role coyotes might play as a reservoir for the parasite.

Three male and three female wild-caught 5 mo old coyotes were used. Coyotes were obtained at 3 wk of age from central and northcentral Oklahoma (35°50′–36°50′N to 97°00′–98°00′W) by

United States Department of Agriculture Animal Control personnel and maintained in captivity. Animals were housed in shaded outdoor cement runs at the Wild Animal Research Facility, Oklahoma State University College of Veterinary Medicine (Stillwater, Oklahoma, USA) and observed daily for changes in attitude, appetite, and body condition. Coyotes were fed dry and canned commercial dog food and water was available ad libitum. Tick control was maintained through monthly application of fipronil (Frontline®, Merial, Iselin, New Jersey, USA). All coyotes were tested by enzyme linked immunosorbent assay (ELISA) for the presence of antibodies to *Ehrlichia canis* and *Rickettsia rickettsii* at the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma). Both assays were performed using the Pan-bio INDX dipstick (Columbia, Maryland, USA). In addition to testing for evidence of exposure to *E. canis* and *R. rickettsii,* the source dog for *B. gibsoni* was also tested for *Dirofilaria immitis* antigen via immunoassay (Snap® test, IDEXX, Westbrook, Maine, USA).

Whole blood was collected in tubes containing potassium ethylenediaminetetraacetic acid (EDTA) from a 9 mo old AST (source dog) from central Oklahoma that was naturally and chronically infected with *B. gibsoni.* Within 3 hr of source blood collection, the six coyotes were sedated via intramuscular injection with a combination of 4 mg/kg ketamine HCl (KetaVed®, Vedco, Inc., St. Joseph, Missouri, USA) and 2 mg/kg xylazine (TranquiVed Injection®, Vedco, Inc., St. Joseph, Missouri) (Kreeger et al., 2002) and physical examination and urine collection were performed. Whole blood and serum were collected from all coyotes and then four (two male, two female) were inoculated intravenously with 3 ml of blood from the AST source dog containing approximately 1×10^6 *B. gibsoni* organisms. Degree of parasitemia was estimated by counting 1,000 red blood cells (RBC) on a Diff-Quik® (Dade Behring, Duluth, Georgia, USA) stained blood film and determining the percent of *B. gibsoni* infected cells. The remaining two coyotes (one male, one female) were controls and received no parasitemic blood. Animals were sedated weekly for the first 8 wk and then monthly for 20 wk post-inoculation in order to obtain body weight, perform physical examinations, and collect urine, whole blood, and serum. Complete blood counts (CBC) were performed on an automated hematology unit (CellDyn 3500, Abbott Diagnostics, Santa Clara, California, USA) validated for veterinary species, and full biochemistry panels were performed on a Vitros 950 analyzer (OrthoClinical Diagnostics, Rochester, New York, USA). Manual white blood cell differential counts were performed, as were manual reticulocyte counts. Manual platelet counts were performed when the automated count was <100,000/μl. Infection status of *B. gibsoni* in coyotes was assessed by polymerase chain reaction (PCR) assay using primers that amplify most of the 18S rRNA gene from the order Piroplasmorida (Kocan et al., 2001) as well as microscopic evaluation of Diff-Quik® stained blood films. Polymerase chain reaction was performed once for each coyote at the height of parasitemia, while stained blood films were examined for every sample of blood collected. DNA was extracted from 200 μl of whole blood with the QIAamp Blood Kit (Qiagen Inc., Valencia, California) according to manufacture's instructions. Polymerase chain reaction amplification was performed as previously described by Kocan et al. (2001). The sequence of the forward primer (BH1-NSSRNA-) was 5′-GTCAGGATCCTGGTTGATCCTGCCAG-3′ and that of the reverse primer (ER1-NSSRNA-3-2) was 5′- GACTGAATTCGACTTCTCCTTCCTTTAAG-3′. Positive control reactions used DNA isolated from whole blood known to carry an Oklahoma isolate of *B. gibsoni.* The negative control was distilled deionized water. Polymerase chain reaction products were purified using the QIAquick PCR Purification Kit (Qiagen Inc.) and sequenced at the Oklahoma State University Recombi-

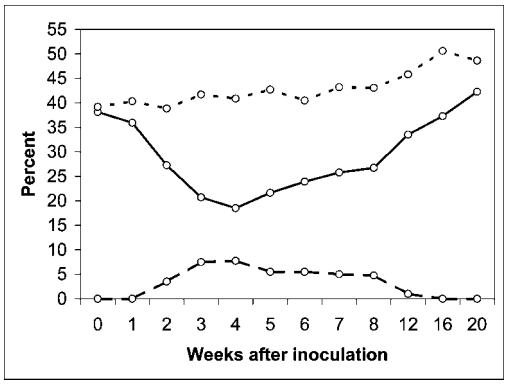

FIGURE 1. Average packed cell volume (PCV; ———○———) and parasitemia (% red blood cells infected with *B. gibsoni;* ———○——— ———○———) for four coyotes experimentally infected with *B. gibsoni* and average PCV (— —○— —) for two *B. gibsoni* negative control coyotes.

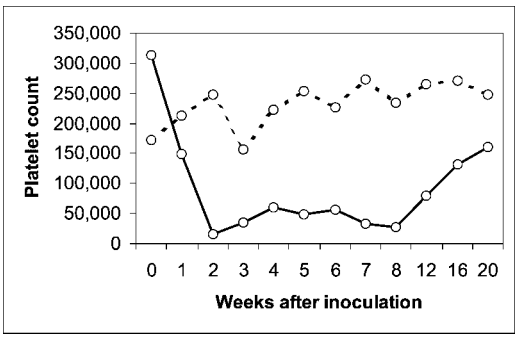

FIGURE 2. Average platelet count (platelets/μl) for four coyotes experimentally infected with *B. gibsoni* (———○———) and two *B. gibsoni* negative control (— —○— —) coyotes.

nant/DNA Protein Research Facility (Stillwater, Oklahoma) using a 373 A automated DNA sequencer (Applied Biosystems, Foster City, California).

Analysis of serum from the AST source dog obtained prior to initiation of the experimental trials was negative for antigens to *D. immitis* and antibodies to *E. canis.* However a weak antibody titer (equivalent to 1:16–128 indirect fluorescent antibody titer) to *R. rickettsii* was detected but was considered not to be indicative of active infection. Prior to experimental exposure, antibodies were not detected in sera of the coyotes for *E. canis* or *R. rickettsii.* Microscopic evaluation of Diff-Quik® stained blood films and PCR analysis demonstrated the source dog was infected with *B. gibsoni* and that before the study the organism was not detected in the coyotes.

All experimentally inoculated coyotes became infected with *B. gibsoni* and infection was confirmed by weekly and monthly microscopic detection of parasites in blood films and PCR analysis at the height of each animal's parasitemia. A partial DNA sequence of 650 bp was obtained from the coyote with the highest parasitemia and found to be 99% similar to the *B. gibsoni* isolate from Oklahoma (GenBank accession number AF205636). *Babesia gibsoni* piroplasms were detectable by microscopic evaluation of stained blood films as early as 1 wk post-inoculation and persisted for at least 20 wk. Parasitemia peaked at 3–4 wk (8–11%), but remained ≥1% for at least 12 wk (Fig. 1). All four experimentally infected coyotes developed a mild elevation in temperature (~1 C, wk 4–12), pale mucous membranes (wk 4–8), and splenomegaly (wk 3–20). A positive heme reaction in the urine was noted 3–16 wk post-inoculation. Mild depression and inappetence were recorded during weeks 3–4 in one male coyote. Regenerative anemia and thrombocytopenia (Fig. 2) were detected that were inversely related to the level of parasitemia (Fig. 1). Packed cell volume (PCV) declined to 16.7–22.5% or $1.84–2.8\times10^6$ RBC/μl (reference range: 33–55% or $5.5–8.5\times10^6$ RBC/μl), reticulocyte numbers peaked at 454,300–590,800/μl (15.4–28.7%) at 4–5 wk. Coyotes also became neutropenic from wk 2–8 (1,520–2,950/μl, reference range: 3,000–12,000/μl); however no significant changes in the biochemistry panel were observed. Two control coyotes remained negative for *B. gibsoni* throughout the study and all evaluated parameters used to measure clinical disease stayed within reference ranges for domestic dogs.

While hematologic evidence of hemolytic disease was found, general body condition of the coyotes did not appear af-

fected by *B. gibsoni* infection. The low morbidity and lack of mortality indicated that experimental infection with *B. gibsoni* did not significantly affect health of these coyotes. However, the long duration and high level of parasitemia suggested that coyotes could serve as potential reservoirs for *B. gibsoni.* Although *B. gibsoni* is a tick-borne parasite, in North America the tick vector for it and other small piroplasms of canids has not yet been identified. Maintenance of the disease almost exclusively in AST colonies suggests that mechanical blood transfer and vertical transmission may be the methods by which *B. gibsoni* is being maintained (Abu et al., 1973; Correa, 1974; Irizarry-Rovira et al., 2001). Finding that coyotes are susceptible to infection and that the disease was relatively mild while maintaining detectable parasitemia indicates that even without a tick vector, interspecies interactions could transmit *B. gibsoni* between wild and domestic canids. In Oklahoma 10–14% of 252 wild canids studies contained domestic dog and coyote traits and 5.6–10.7% contained red wolf (*Canis rufus*) and coyote traits (Freeman and Shaw, 1979). While these numbers give some indication of the frequency of interspecies breeding, it is likely that fighting and other opportunities for blood transfer between species also occurs.

Discovery that coyotes, a well distributed and commonly occurring wild canid, are susceptible to infection with *B. gibsoni* coupled with recent entry and establishment of this parasite in domestic dogs in North America supports the need for more stringent evaluation of canines entering and re-entering the United States. While quarantine periods are beneficial, complete physical exams and microscopic evaluation of stained whole blood should also be performed to reduce the risk of foreign animal diseases entering the United States.

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