Seroprevalence of antibodies against *Leishmania* spp among dogs in the United States

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**Objective**—To determine seroprevalence of antibodies against *Leishmania* spp among dogs other than Foxhounds in the United States.

**Design**—Cross-sectional study.

**Sample Population**—957 serum samples from dogs throughout the United States submitted between January 2000 and August 2001 to the Diagnostic Center for Population and Animal Health at Michigan State University for serologic testing for tick-borne diseases.

**Procedure**—Samples were tested for antibodies against *Leishmania* spp with an immunofluorescent antibody (IFA) assay. Samples with positive results were submitted to the Centers for Disease Control and Prevention for confirmatory testing.

**Results**—Results of the IFA assay were negative for 939 of 957 samples. For 16 samples, titers were from 1:16 to 1:64, and titers in these dogs were considered likely to be a result of cross-reactivity with antibodies directed against other organisms. For the remaining 2 samples, the titers were ≥ 1:128. One of these samples was from a blood donor dog that had never had any clinical signs of leishmaniasis. Follow-up samples from both dogs also had *Leishmania* IFA titers ≥ 1:128. Both dogs had antibodies against *Trypanosoma cruzi*, as determined with a radioimmunoprecipitation assay.

**Conclusions and Clinical Relevance**—Results suggested that the seroprevalence of antibodies against *Leishmania* spp in dogs in the United States was low. However, results further suggested that leishmaniasis may not be limited to Foxhounds in the United States. (J Am Vet Med Assoc 2003;222:603–606)

Leishmaniasis is a rare, potentially zoonotic disease caused by protozoal organisms of the genus *Leishmania*. Several forms of the disease, including cutaneous, mucocutaneous, and visceral forms, have been identified in dogs. Visceral leishmaniasis is a chronic systemic disease that affects the kidney, liver, spleen, lymph nodes, and bone marrow; and the causative organism, *Leishmania donovani*, is most commonly found in tropical and subtropical areas of the world. Sand flies (*Phlebotomus* spp or *Lutzomyia* spp) are currently the only known vectors for *Leishmania* organisms; and although there are sand flies in the southern United States, no infected sand flies have been reported in the United States to date. Current investigations have suggested that direct transmission or iatrogenic transmission through blood is possible.

The first confirmed case of visceral leishmaniasis in a dog in the United States was identified in 1980 and involved a dog from Oklahoma. Since then, cases have been reported from 21 states, including Ohio, Michigan, Texas, New York, and Alabama, and it appears that the incidence of visceral leishmaniasis among dogs in the United States may be increasing. To date, most affected dogs have been Foxhounds, particularly Foxhounds kenneled at hunt clubs. The purpose of the study reported here was to determine seroprevalence of antibodies against *Leishmania* spp among dogs other than Foxhounds in the United States.

**Materials and Methods**

**Sample population**—All serum samples tested in the present study had been submitted to the Diagnostic Center for Population and Animal Health (DCPAH) for tick-borne disease testing, which included immunofluorescent antibody (IFA) assays for antibodies against *Borrelia burgdorferi*, *Babesia canis*, *Ehrlichia canis*, *E risticii*, and *Rickettsia rickettsii*.

All samples were submitted between January 2000 and August 2001 and stored frozen immediately after testing at –20°C until used in the present study. The age, breed, and location of the dogs were documented when possible. None of the dogs for which breed was identified were Foxhounds. The specific breed was not identified for 361 of the 937 samples. None of the dogs included in the study were known to be Foxhounds, but Foxhounds were not specifically excluded.

**Study protocol**—All serum samples were tested by the DCPAH for antibodies against *Leishmania* spp with an IFA assay incorporating the DD8 strain of *L. donovani*. The organism was obtained from the Centers for Disease Control and Prevention (CDC) in November 2000 and cultured in Noyv-MacNeal-Nicolle medium, as described. Cultures were transferred to fresh medium on a weekly basis. To prepare antigen for the IFA assay, 10 to 15 mL of a 3-day-old culture was placed in a 50-mL conical tube and centrifuged at 1,877 X g for 10 minutes. The supernatant was discarded, and the pellet was reconstituted in 10 mL of phosphate-buffered saline solution (PBSS) and centrifuged again. Four additional washes were performed with PBSS, and the final pellet was reconstituted in 30 mL of PBSS. The
Slides were air dried and stored at –80°C until used. For the IFA assay, all samples were initially tested at a dilution of 1:16. For samples with positive results at a dilution of 1:16, serial 2-fold dilutions were tested until an endpoint titer was reached. Appropriate positive and negative controls were included on each slide.

Confirmatory testing—Samples from dogs with Leishmania IFA assay titers ≥ 1:128 were submitted to the CDC for confirmatory testing by means of IFA testing and culture. The IFA assay was performed as described for the IFA assay performed by the DCPAH. For culture, blood samples (2 to 3 mL) were centrifuged, and the packed RBCs and Buffy coat layer were removed and placed in Novy-MacNeal-Nicolle medium with an RPMI overlay. Cultures were maintained for 5 weeks. If no promastigotes were seen after 5 weeks, results were considered negative.

Samples submitted to the CDC were also tested with an IFA assay for antibodies against Trypanosoma cruzi, because cross-reactions between antibodies directed against these organisms have been reported. Samples were initially tested at a dilution of 1:16. For samples with positive results at a dilution of 1:16, serial 2-fold dilutions were tested until an endpoint titer was reached. The strain used as antigen for the T cruzi IFA assay was the Y strain; organisms were in the epimastigote stage of development.

Samples submitted to the CDC that yielded positive Leishmania IFA assay or Trypanosoma IFA assay results were submitted to the University of Iowa for further testing. At the University of Iowa, a radioimmunoprecipitation assay (RIPA) was performed to detect antibodies against T cruzi. Antibodies against Leishmania spp and T cruzi reportedly do not cross-react in the RIPA.

Amplification of Leishmania DNA—Blood samples were collected from dogs with high Leishmania IFA titers. The DNA was extracted with a commercial kit and used in polymerase chain reaction (PCR) assays developed to detect DNA from Leishmania spp, T cruzi, and B canis. For the Leishmania PCR assay, primers designed to amplify a kinetoplastid-specific central portion of the SSU rRNA gene were used. For the Trypanosoma PCR assay, primers designed to amplify specific kDNA minicircle fragments from T cruzi cells were used. For the Babesia PCR assay, primers developed by Kordick et al were used.

Results—A total of 957 serum samples submitted to the DCPAH during the study period were tested for antibodies against Leishmania spp with the IFA assay. Dogs from which samples were obtained represented 80 breeds and ranged from 6 months to 16 years old. None of the dogs were known to be Foxhounds. One hundred forty-five samples were from dogs being used as blood donors.

Results of the Leishmania IFA assay were negative for 939 of the 957 serum samples. Sixteen samples yielded titers between 1:16 and 1:64. These titers were assumed to be a result of cross-reaction with other antibodies and were not considered to be an indication of exposure to Leishmania spp.

The remaining 2 samples yielded Leishmania IFA titers ≥ 1:128. One of these samples was from a 2-year-old male Bullmastiff (dog 1) from Texas. This dog had no history of traveling outside the United States. A serum sample had initially been submitted to the DCPAH for testing for tick-borne diseases in May 2000, prior to enrollment of the dog in a blood donor program. Results of testing for antibodies against B canis (1:80) were considered positive, results of testing for antibodies against B burgdorferi (1:160) were considered suspect, and results of testing for antibodies against E canis (1:20), E risticii, and R ricketttsii were considered negative. Because the dog did not have any clinical signs of disease, the owners assumed these results were not accurate and sent another serum sample to a diagnostic laboratory in Texas. At the Texas laboratory, results of IFA assays for antibodies against B burgdorferi, B canis, E canis, and B gibsoni were negative. A second sample was sent to the laboratory in Texas and, again, results were negative. Therefore, the dog was enrolled in the blood donor program.

The serum sample submitted to the DCPAH in May 2000 was tested for antibodies against Leishmania spp in August 2001 and yielded a titer of 1:1,024. The sample was sent to the CDC for confirmatory testing. At the CDC, a Leishmania IFA assay yielded a titer of 1:512, and a Trypanosoma IFA assay yielded a titer of 1:1,024. The sample was sent to the University of Iowa for additional testing, and results of the RIPA for antibodies against T cruzi were positive.

The veterinarian caring for the dog was notified of the test results, and the dog was immediately removed from the blood donor program. Blood and serum samples collected 15 months after the initial serum sample were submitted for testing. At the DCPAH, a Leishmania IFA assay yielded a titer of 1:512. At the CDC, a Leishmania IFA assay yielded a titer of 1:256, and a Trypanosoma IFA assay yielded a titer of 1:512; results of culture for Leishmania organisms were negative. At the University of Iowa, results of the RIPA for antibodies against T cruzi were positive. Results of PCR assays for Leishmania, Trypanosoma, and Babesia DNA were negative.

Two dogs from the same household as dog 1 were tested for antibodies against Leishmania spp with the IFA assay. Titers were 1:16 and 1:32. The second dog with a Leishmania IFA titer ≥ 1:128 was a 2.5-year-old neutered male Greyhound (dog 2) from New York. The dog’s travel history was unknown, because it had been obtained from a racetrack. In April 2001, the dog had clinical signs compatible with a tick-borne infection or leishmaniasis. Clinical signs included limping, pitting edema of the right hind limb, and fever. The dog was treated with doxycycline (300 mg, PO, q 24 h) for 3 weeks and prednisone (20 mg, PO, q 12 h) for 10 days and improved clinically. Results of testing for antibodies against B canis (1:160) were considered positive, results of testing for antibodies against R ricketttsii were negative, and results of testing for antibodies against T cruzi were negative.
(1:40) were considered suspect, and results of testing for antibodies against *B. burgdorferi*, *E. canis*, and *E. risticii* were considered negative.

The serum sample submitted to the DCPAH in April 2001 was tested for antibodies against *Leishmania* spp in August 2001 and yielded a titer of 1:128. The sample was sent to the CDC for confirmatory testing. At the CDC, a *Leishmania* IFA assay yielded a titer of 1:256, and a *Trypanosoma* IFA assay yielded a titer of 1:1,024. Results of the RIPA performed at the University of Iowa were positive.

Blood and serum samples collected 4 months after the initial serum sample were submitted for testing. At the DCPAH, a *Leishmania* IFA assay yielded a titer of 1:128. At the CDC, a *Leishmania* IFA assay yielded a titer of 1:256, and a *Trypanosoma* IFA assay yielded a titer of 1:512; results of culture for *Leishmania* organisms were negative. At the University of Iowa, results of the RIPA for antibodies against *T. cruzi* were positive. Results of PCR assays for *Leishmania*, *Trypanosoma*, and Babesia DNA were negative.

To ensure that results of the *Leishmania* IFA assay performed at the DCPAH were comparable to results of the assay performed at the CDC, *Leishmania* IFA results from the DCPAH and the CDC were assessed for agreement with a $\kappa$ statistic weighted for ordinal categories. The weighted $\kappa$ value for the *Leishmania* IFA test was 0.59 (95% confidence interval = 0.42 to 0.77). This was considered moderate (0.41 to 0.60 by Landis and Koch9) or good reproducibility (0.4 to 0.75 by Rosner10).

**Discussion**

Results of the present study suggest that the seroprevalence of antibodies against *Leishmania* spp in dogs in the United States is low. However, results further suggest that veterinarians, particularly veterinarians associated with blood donor programs, should be aware that leishmaniasis may not be limited to Foxhounds in the United States. In the present study, 2 of 957 serum samples were positive for antibodies against *Leishmania* spp, as determined with an IFA assay. One of these serum samples was from a dog that had been used as a blood donor, and this dog reportedly had not ever had any clinical signs compatible with leishmaniasis.

It is impossible to determine definitively on the basis of results of tests performed in the present study whether dogs 1 and 2 were infected with *Leishmania* spp. Results of *Leishmania* PCR assays performed on blood samples from both dogs were negative, and the limit of detection with this assay is approximately 10 organisms.1 Organisms are most often identified in bone marrow, spleen, and lymph nodes.2 Although results of the *Leishmania* IFA assays performed at the DCPAH and the CDC on serum samples from these dogs were positive, this assay only detects antibodies directed against the organism and may be falsely positive because of cross-reaction with antibodies against *T. cruzi*.3 Culture of a bone marrow aspirate is the most definitive test for diagnosing leishmaniasis; however, owners of both dogs declined this procedure.

It is likely that both of these dogs were infected with *T. cruzi*, because the RIPA detects antibodies specific for *T. cruzi* glycoproteins and is not subject to false-positive results associated with cross-reactivity to antibodies against *Leishmania* spp. In addition, dog 1 was from Texas where *T. cruzi* has been identified. However, dog 1 reportedly did not ever have any clinical signs of trypanosomiasis, and clinical signs in dog 2 were more consistent with leishmaniasis than with trypanosomiasis. Common clinical signs in dogs with leishmaniasis are typically nonspecific and include skin lesions, listlessness, fever, weight loss, epistaxis, lymphadenopathy, hepatomegaly, splenomegaly, renal failure, and swollen limbs and joints,4 whereas clinical signs of trypanosomiasis include pale mucous membranes, slow capillary refill time, weak pulses, pulse deficits, tachyarrhythmias, hypothermia, and respiratory tract disease.5 Regardless, we cannot rule out the possibility that these 2 dogs were coinfected with *Leishmania* spp and *T. cruzi*.

Both dogs 1 and 2 described in the present report also had antibodies against *B. canis*, as determined by means of the IFA assay. However, results of the Babesia PCR assay were negative for both dogs. Together, these results suggest that both dogs had been exposed to *B. canis*, but that neither was carrying the organism at the time samples were collected. Alternatively, it is possible that the *B. canis* titers were a result of cross-reaction with antibodies against *Leishmania* spp or *T. cruzi*. However, in our laboratory, testing of 46 serum samples with *B. canis* titers $\geq 1:80$ with the *Leishmania* IFA assay yielded only 4 *Leishmania* suspect titers. Thus, it seems unlikely that antibodies against *Leishmania* spp cross-react extensively with *B. canis*. It is not known whether antibodies against *T. cruzi* cross-react with *B. canis* in the IFA assay.

Greyhounds and other large-breed dogs are commonly used as blood donors in the United States. Results of the present study suggest that all potential donors should be tested for antibodies against *Leishmania* spp and *T. cruzi*, along with other agents, prior to enrollment in a blood donor program. In addition, data documenting the dog's travel history should be obtained, but should not be considered a substitute for serologic testing.

**References**


6. van Eys GJ, Schoone GJ, Kroon NCM, et al. Sequence analy-


