Leishmaniasis in a dog native to Colorado

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Case Description—A 1-year-old 32.5-kg (71.5-lb) sexually intact male foxhound-Treeing Walker Coonhound cross was evaluated because of a 2.5-month history of dermatologic lesions, weight loss, and diarrhea.

Clinical Findings—Physical examination revealed muscle wasting, lymphadenopathy, and multifocal pruritic dermatologic lesions of alopecia, thickening, erythema, and follicular casting. Hematologic and serum biochemical analyses revealed nonregenerative anemia, monocytosis, hypercalcemia, hyperproteinemia, and hyperglobulinemia. Proteinuria was identified on urinalysis. Hepatomegaly, splenomegaly, and diffuse abdominal lymphadenomegaly were detected on abdominal ultrasonography. A diagnosis of leishmaniasis was confirmed by ELISA detection of serum antibodies against Leishmania spp. A high serum indirect fluorescent antibody titer (1:1,024) against Leishmania infantum, amplification of Leishmania DNA on PCR assay of a whole blood sample and a lymph node aspirate, and histologic identification of suspected Leishmania amastigotes in skin specimens. In addition, the dog had a low CD4+:CD8+ lymphocyte ratio of 1:1.

Treatment and Outcome—The dog was euthanized because of the severity of leishmaniasis and poor prognosis. This dog was from a litter of 10 puppies that included 4 stillborn puppies, 2 puppies that died as neonates, and 1 littermate that was euthanized at 1 year of age because of a high serum antibody titer against Leishmania spp. Eventually the foxhound dam was euthanized because of a high serum antibody titer against Leishmania spp. The dog had been raised with an unaffected littermate, its sire, and an unrelated Treeing Walker Coonhound female that were seronegative for Leishmania infection.

Clinical Relevance—Although vertical disease transmission was suspected, it is possible that L. infantum is now endemic in Colorado. Leishmaniasis should be considered in dogs with scaly dermatoses. (J Am Vet Med Assoc 2010;237:1288–1291)

A 1-year-old 32.5-kg (71.5-lb) sexually intact male foxhound-Treeing Walker Coonhound cross with no travel history outside of Colorado was admitted to the Colorado State University Veterinary Teaching Hospital in the fall of 2008. The dog was examined because of a 2.5-month history of diarrhea, progressive weight loss despite a good appetite, dermatologic lesions, and hind limb stiffness. In an effort to control the diarrhea, which appeared to commence after a food change, at least 2 diet trials (including 1 raw meat type) were fed over the 2.5 months prior to admission at the veterinary teaching hospital, with no notable change in the diarrhea. Fecal flotation testing in early October revealed Giardia spp. cysts, and metronidazole (23 mg/kg [10.5 mg/lb], PO, q 12 h for 8 days) and pyrantel pamoate (10.8 mg/kg [4.9 mg/lb], PO, once) were administered. The diarrhea improved slightly after these treatments, but returned. After the raw food trial was finished, bacterial culture of feces was found to be negative for Salmonella spp. Additionally, prior to evaluation at the veterinary teaching hospital, serum biochemical profile revealed that the dog was hyperproteinemic (9.1 mg/dL; reference range, 5.2 to 8.2 mg/dL), was hyperglobulinemic (6.3 mg/dL; reference range, 2.5 to 4.5 mg/dL), and had a high BUN concentration (39 mg/dL; reference range, 7 to 27 mg/dL).

Dermatologic lesions were initially nonpruritic and began as an alopecic area on the crown of the head. Over approximately 8 weeks, the lesions progressed to become erythematous and pruritic, with scaling involving the face, muzzle, and axillary regions. Weeks prior to evaluation at the veterinary teaching hospital, a skin scraping was negative for Demodex mites on microscopic examination and had negative dermatophyte culture results. A 7-day trial treatment with oral administration of ivermectin once a day (day 1, 0.08 mg/kg [0.03 mg/lb]; day 2, 0.15 mg/kg [0.07 mg/lb]; days 3 through 7, 0.23 mg/kg [0.1 mg/lb]) did not elicit improvement in the skin lesions. Twelve days after completion of ivermectin administration, a skin biopsy specimen was submitted to the Colorado State University Veterinary Diagnostic Laboratory; histologic evaluation revealed severe chronic folliculitis, potentially of bacterial origin, but no organisms were observed. Cephalexin (23 mg/kg, PO, q 12 h) was administered for 30 days with minimal clinical response prior to referral to the veterinary teaching hospital.

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At the time of referral, the dog was bright, alert, and responsive, but was thin with a body condition score of 2.5/9. There was mucopurulent discharge from the left eye and bilateral blepharitis. The dog had generalized muscle wasting, peripheral lymphadenopathy, and pruritic lesions of diffuse thickening, alopecia, moderate erythema, and follicular casting and crusting on the cranium, face, pinna, ventrum, axillary, and inguinal regions. Palpation suggested bilateral tarsal joint effusion, and the left tarsus seemed painful.

Initial diagnostic tests included a CBC, serum biochemical profile; urinalysis; cytologic examination of fine needle aspirates of the prescapular lymph nodes; serologic testing for *Dirofilaria immitis* antigen and antibodies against *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi*; trypsin-like immunoreactivity assay; microscopic examination of a skin scraping from an affected area on the calvarium; dermatophyte culture of hair collected from multiple regions; and abdominal ultrasonography.

Hematologic and serum biochemical analyses revealed that the dog had a nonregenerative anemia (PCV, 32%; reference range, 40% to 55%), monocytosis (1.4 X 10³ cells/µL; reference range, 0.2 X 10³ cells/µL to 1.0 X 10³ cells/µL), mild hypercalcemia (11.8 mg/dL; reference range, 9.2 to 11.7 mg/dL), hyperproteinemia (9.0 g/dL; reference range, 5.3 to 7.2 g/dL), and hyperglobulinemia (6.3 mg/dL; reference range, 2.0 to 3.8 g/dL). Urinalysis revealed that the dog had proteinuria and a urine specific gravity of 1.015. A cytologic examination of fine needle aspirates of the prescapular lymph nodes revealed reactive lymphocytes. Trypsin-like immunoreactivity was 9.6 µg/L (reference range, 5.7 to 45.2 µg/L). Results of a serologic test for detection of *D immitis* antigen were negative, and serum antibodies against *E canis*, *A phagocytophilum*, and *B burgdorferi* were not detected. Results of fungal culture and microscopic examination of skin scrapings were negative. Findings on abdominal ultrasonography included mild hepatomegaly, mild splenomegaly, diffuse abdominal lymphadenomegaly, a possible retained testis in the right side of the abdomen, and a thickening of the wall of the cranioventral portion of the urinary bladder.

Despite the fact that *Leishmania* spp amastigotes were not found on microscopic examination of multiple skin scrapings, on cytologic examination of fine-needle aspirates of the prescapular lymph nodes, or on histologic examination of skin biopsy specimens, the breed, clinical findings, and hematologic abnormalities were consistent with leishmaniasis. Thus, a serum sample was submitted for detection of antibodies against *Leishmania* spp. While awaiting results, the dog was discharged from the hospital and prescribed tylosin (10.6 mg/kg [4.8 mg/lb], PO, q 8 h) for 14 days and a probiotic in an effort to ameliorate the diarrhea, in addition to the previously prescribed cephalaxin.

After 10 days of treatment, the diarrhea had not resolved, and the dog continued to lose weight despite a good appetite. Serum antibodies against the *Leishmania* recombinant antigen K39, which is specific for *Leishmania donovani* complex, were detected. Whole blood and popliteal lymph node aspirates were submitted for a PCR assay of *Leishmania* DNA, and a serum sample was submitted for indirect fluorescent antibody testing for antibodies against *Leishmania* spp. The dog had a high serum indirect fluorescent antibody titer (1:1,024) against *Leishmania infantum*, and DNA specific for *Leishmania* spp was amplified in a whole blood sample and a popliteal lymph node aspirate via PCR assay, confirming the diagnosis of leishmaniasis. The owners declined treatment because of the dog’s working status, the severity of the disease, and the expense of treatment with little chance of a cure.

The dog was euthanized by IV administration of an overdose of thiopental and pentobarbital, and a necropsy was performed. Histologic examination of skin specimens revealed chronic perifolliculitis, which was lymphohistiocytic and plasmacytic with multifocal to coalescing lesions. Intrahistiocytic amastigotes were observed, consistent with *Leishmania* organisms found in the haired skin. Membranoproliferative mesangial glomerulonephritis, with moderate to marked lymphoplasmacytic interstitial nephritis, portal hepatitis, and diffuse bone marrow hyperplasia, was reported. Although the *Leishmania* amastigotes were found in the skin, they were not found in other tissues.

While the dog in this report was in utero, its foxhound dam had clinical manifestations consistent with leishmaniasis; in the summer of 2007, the foxhound dam was pregnant and had been brought to the veterinary teaching hospital for evaluation of epistaxis. On physical examination, lymphadenopathy was found, and approximately 11 fetuses were seen ultrasonographically. Serum biochemical profile and CBC revealed that the dam had eosinophilia (1.7 X 10³ cells/µL; reference range, 0.1 X 10³ cells/µL to 1.2 X 10³ cells/µL), regenerative anemia (PCV, 22%; reference range, 40% to 55%), hyperproteinemia (9.3 g/dL; reference range, 5.3 to 7.2 g/dL), and hyperglobulinemia (7.0 g/dL; reference range, 2.0 to 3.8 g/dL). Prothrombin and partial thromboplastin times were within reference limits. Results of a serologic test for *D immitis* antigen were negative, and serum antibodies against *E canis*, *A phagocytophilum*, and *B burgdorferi* were not detected. Fenbendazole (51.4 mg/kg [23.4 mg/lb], PO, q 24 h) was administered for 3 days and doxycycline (9.2 mg/kg [4.2 mg/lb], PO) was administered once prior to the determination that the dam was seronegative for *E canis*. At whelping, 4 puppies were stillborn, 1 puppy died within a week, and 1 died within a few weeks, and 4 puppies, which included the dog of this report, were seemingly healthy. The 4 stillborn puppies were necropsied at Colorado State University Veterinary Diagnostic Laboratory and found to be full-term males with lungs that appeared to have never been expanded with air. Severe autolysis was found in all stillborn puppies with the addition of splenomegaly, hepatomegaly, and renomegaly in 1 puppy; blood clots on the dorsal surface of the brain in 1 puppy; and petechial hemorrhages throughout the thymic parenchyma of 1 puppy. Histologic evaluation of affected tissues was not performed because of the degree of autolysis; therefore, no paraffin-embedded tissues were available for subsequent PCR assay of *Leishmania* DNA.

The dam was from a foxhound kennel club in Kansas and was bred in Colorado with a previously healthy...
Treeing Walker Coonhound from Colorado. The foxhound dam had lived primarily in Kansas, but had also traveled to foxhound kennel clubs in Michigan and South Carolina, all 3 states of which are included in the 21 states where the CDC has found serologic evidence of *Leishmania* exposure in hunting dogs.\(^2\) Colorado, however, is not one of the states where either the CDC or a recent United States and Canadian seroprevalence study\(^1\) has found *Leishmania* spp in the canine population. The foxhound dam lived in Colorado for 8 months in 2007. Although her epistaxis reportedly resolved, she was euthanized about 8 months after returning to Kansas because of a high serum antibody titer for *Leishmania* spp.

Of the 4 puppies that survived, 3 (all male) remained in Colorado and 1 (female) returned with the dam to Kansas. The female puppy was euthanized at 1 year of age when a high serum antibody titer against *Leishmania* spp was detected. Of the 3 male littermates that never left Colorado, the clinically ill dog described in this report lived with 1 unaffected male littermate, and the other unaffected male littermate lived in a separate residence in the same neighborhood. The affected and unaffected littermates that lived together in Colorado also lived with their Treeing Walker Coonhound sire and an unrelated Treeing Walker Coonhound female. After the diagnosis of leishmansionasis was confirmed in the dog of this report, tests, which included PCR assays for detection of *Leishmania* DNA in whole blood samples and lymph node aspirates, indirect fluorescent antibody testing for detection of serum antibodies against *Leishmania* spp, and flow cytometry\(^4\) for determination of lymphocyte subset distributions (CD4+ and CD8+) in blood samples, were performed for all 4 dogs of the household. Flow cytometry results revealed a low CD4+/CD8+ lymphocyte ratio in the clinically affected male (420 CD4+ and 406 CD8+ [1:1 ratio]), compared with the unaffected littermate (649 CD4+ and 317 CD8+ [2:1 ratio]) and the 2 older Treeing Walker Coonhounds (309 CD4+ and 98 CD8+ cells/μl [3:1 ratio] and 693 CD4+ and 139 CD8+ [5:1 ratio]). On the basis of the negative serologic test results and PCR assay results, the sire, unaffected male littermate, and unrelated bitch did not appear to be infected by *Leishmania* spp at that time. However, it is important to note that because of the latent period, serum antibodies may not be detectable in *Leishmania*-infected dogs prior to the onset of clinical disease.\(^4\) This may have occurred in the unaffected 2.75-year-old male littermate and housemate that developed weight loss despite a good appetite and end-stage renal disease leading to euthanasia nearly 2 years after the dog of this report was determined to have leishmansionasis. However, diagnosis of leishmansionasis in this dog is unconfirmed, as necropsy and PCR testing were not performed.

**Discussion**

Leishmansionasis is a disease caused by a zoonotic protozoal parasite that is frequently transmitted by sandflies in the Mediterranean basin, India, and South America. Until recently, leishmansionasis has been rarely reported for dogs indigenous to North America, and to date, no autochthonous cases of visceral leishmansionasis in humans have been reported from the United States. Transmission of *Leishmania* spp among canids outside of the United States is known to be vector borne via sandflies (eg, *Lutzomyia* spp or *Phlebotomus* spp). Within the United States, no definitive vector for transmission of *Leishmania* spp has been identified. The sandfly species identified to transmit visceral leishmansionasis (*Leishmania chagasi*) in South America have not been found in North America.\(^9\) There are at least 4 sandfly species in North America that are found in regions where leishmansionasis outbreaks in foxhound kennels have occurred.\(^6\) In an experimental study,\(^7\) *Leishmania* amastigotes were found via microscopic evaluation in a sandfly species (*Lutzomyia shannoni*) that was feeding on an infected dog in South America. Although the sandflies were from South America, *L. shannoni* is a sandfly species also found in the United States. In a recent study,\(^8\) *Rhipicephalus sanguineus* (the brown dog tick) was found to be susceptible to *L. chagasi* when placed on infected dogs; this research revealed that the ticks were able to transmit the parasite to an experimental host. Additionally, *Leishmania* spp have been shown experimentally to be transmitted from infected to noninfected dogs via *R. sanguineus*.\(^6\) Despite experimental evidence that *Leishmania* can be transmitted via various vectors in the United States, none of these vectors are known to have infected animals during US outbreaks. Also, while vector transmission is clearly an important aspect of the *Leishmania* protozoal life cycle, vector exposure does not account for all cases of leishmansionasis in the United States and other nonindigenous regions of the world.

While the mode of *Leishmania* transmission for the dog in this report was not definitively established and we cannot entirely rule out vector-borne transmission, vertical transmission was suspected as the dam appeared to have been infected with a *Leishmania* sp during gestation. The types of vertical transmission can include both transplacental and transmammary, and discerning between the 2 is often difficult. There is controversy over the existence of vertical transmission, with 1 study\(^9\) involving 63 puppies born to 18 infected bitches finding no evidence of vertical transmission. In contrast, experimentally, transplacental transmission of *L. infantum* has been demonstrated in laboratory Beagles,\(^10\) and natural vertical transmission of *L. infantum* was presumptively documented by detection of *Leishmania* DNA on PCR assay in samples of whole blood or lymph nodes from 8 of 31 puppies born to 7 infected bitches.\(^11\) While not definitive, further evidence for potential vertical transmission is found in a recent case report\(^12\) of 2 affected 19-month-old foxhounds born to a dam seropositive for *L. infantum*. Additionally, sexual transmission has been an area of controversy, with *L. infantum* shown to be transmitted sexually from infected male mice to uninfected female mice.\(^12\) Additionally, a recent study\(^13\) found evidence of sexual transmission of *L. chagasi* from infected male dogs to noninfected females. Lastly, other methods of reported transmission include direct contact as well as blood transfusion.\(^10,15,16\)

Within the United States, leishmansionasis appears to have a natural tropism, or foxhounds potentially have
genetic predisposition for the disease. In fact, the index case indicating the disease was endemic in dogs in the United States was first reported in 1980 in a foxhound.17 Whether leishmaniasis in foxhounds is related to exposure or an immunologic characteristic of the breed is undetermined. Findings from immunologic testing have revealed that Leishmania-infected dogs have a weak cellular immune response as evidenced by low CD4+ lymphocyte numbers and low CD4+/CD8+ lymphocyte ratios, compared with noninfected dogs.18 Additionally, research comparing the cellular and humoral immune responses in Leishmania-infected dogs with and without clinical signs of disease found that dogs with weaker cellular-mediated immunity are more likely to develop clinical signs of leishmaniasis than dogs with stronger cellular-mediated immune responses.19 Flow cytometry results of the dogs in this report revealed a low CD4+/CD8+ lymphocyte ratio in the affected male, compared with that of the unaffected littermate and the 2 older Treeing Walker Coonhounds. The importance of this low CD4+/CD8+ lymphocyte ratio in the dog of this report is not known, but it does support findings of the previous studies.18,g

To our knowledge, this is the first report of leishmaniasis in a dog that was bred and born in Colorado with no travel history outside of the state. In conjunction with previous observations, findings in the dog of this report provide interesting questions regarding the ongoing transmission and potential spread of Leishmania in dogs in the United States. On the basis of findings in this report, leishmaniasis should be included on the differential list for all dogs with scaly dermatoses, regardless of geographic location within the United States.

References