Placentitis associated with leishmaniasis in a dog

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Leishmania spp are flagellated protozoan parasites that are transmitted by the bite of infected female sand flies.

Leishmaniasis is becoming endemic in Foxhounds in North America.

Leishmania parasites can be transmitted by blood transfusion and by packed RBC transfusion in Foxhounds in North America.

Congenital transmission of visceral leishmaniasis has been reported in humans, but this mode of transmission is uncertain in dogs.

A 1.5-year-old Coonhound was evaluated on an emergency basis at the Animal Medical Hospital at Glenwood after aborting 3 puppies during the previous 12 hours. No vaccination history was known, but the dog had not traveled outside the country. The owner acquired the dog from a kennel in Tennessee, approximately 1 year previously. The dog had been returned to the kennel to be bred. There were no clinical signs or problems noted prior to the abortion.

The dog appeared to be clinically normal except for a green and red vaginal discharge, and puppies were palpable in the abdomen. Cytologic examination of a vaginal smear revealed large numbers of bacterial rods and degenerating neutrophils. Radiography revealed 4 fetuses; 2 fetuses had opacity apparently caused by gas, and 1 fetus was macerated. The dog was hospitalized and administered 5 mg of dinoprost tromethamine, IM, on day 1 to induce abortion of the remaining fetuses. The dog passed a large quantity of black, green, and red fluid from the vagina but no fetuses. On day 2, 1.25 mg of dinoprost tromethamine was given IM at 9 AM and 1:30 PM. At 4:45 PM, the dog was treated with 37.5 mg of carprofen and 1,200 mg of sulfadimethoxine-ormetoprim. At 6 PM, 4 mg of dinoprost tromethamine was given IM. Vaginal swab specimens for bacteriologic culture were also submitted on day 2. No puppies were passed on day 2. On days 3, 4, and 5, treatment continued with administration of 4 mg of dinoprost tromethamine in the morning, afternoon, and evening; carprofen every 12 hours; and sulfadimethoxine-ormetoprim (600 mg) once daily. In the evening on day 5, bones were found in feces. On the morning of day 6, abdomin al radiography was performed and only 1 puppy was seen in the uterus. At this time, it was assumed that the dog had passed the other puppies and consumed them before staff had noticed them. On day 6, treatment continued and the last puppy was passed in the afternoon. The puppy and placenta were submitted to the Maryland Department of Agriculture, Animal Health Laboratory, Frederick, Md, for necropsy examination and viral isolation because the result of the Brucella test was negative and bacteriologic culture grew only Pasteurella multocida and Staphylococcus intermedius from the vaginal specimen.

A necropsy examination was made. The fetus weighed 272 g (0.6 lb) and had a 16.5-cm crown-rump length. The placenta was up to 2 cm thick and congested; the villous surface had focal areas of discoloration caused by necrosis. The fetal tissues were mildly autolyzed, but there was no evidence of maceration. Tissues were fixed in neutral-buffered 10% formalin and processed for routine histologic examination. Paraffin-embedded tissues were cut 5 µm thick and examined after staining with H&E.

Because protozoanlike structures were seen in histologic sections of placenta, samples of placental tissue and fetal organs in paraffin blocks or histologic sections were submitted to the Animal Parasitic Diseases Laboratory, USDA, Beltsville, Md, for further diagnosis. At the Animal Parasitic Disease Laboratory, paraffin-embedded sections were examined immunohistochemically with rabbit polyclonal antibodies against Toxoplasma gondii and Neospora caninum as described. Additional immunohistochemical staining was performed with anti-Leishmania infantum serum as primary antibody. A serum sample was obtained from a rabbit that was inoculated SC with 10⁶ L infantum Virginia Tech-1 isolate promastigotes and that received a booster vaccination 1 month later. The anti-L. infantum serum was diluted 1:400, and sections from a dog naturally infected with L. infantum were included as positive controls in the test. For a negative control, preimmune serum from the rabbit was used at a dilution of 1:100. For immunohistochemical staining, a peroxidase-based anti-rabbit kit was used according to the manufacturer’s recommended procedures. Briefly, after deparaffinization, sections were treated with quenching solutions (3% hydrogen peroxide in absolute methanol), rinsed with water, digested in 0.4% pepsin, rinsed in blocking solution (0.5% casein), and treated with primary antibody at 21°C for 30 minutes. Sections

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were then rinsed and treated for 30 minutes with horse-
radish peroxidase solution from the kit, rinsed with
buffer, treated with substrate 3 amino-9-ethylcarbazole,
rinsed with buffer, counter-stained with Mayer hema-
toxin, and examined microscopically after covering
with mounting solution and coverslips. For electron
microscopy, a sample of placental tissue from the paraf-
fin block was deparaffinized, postfixed in osmium, and
processed for routine transmission electron microscopy.

Bacteriologic examination of placental and fetal
tissues included routine culture on blood agar, choco-
late agar, MacConkey agar, and thioglycolate broth of
fetal lung, liver, and stomach contents; bacteriologic
culture of placenta was also performed. Bacteriologic
culture revealed heavy growth of *Staphylococcus xylo-
sus* from the liver and moderate growth of mixed bac-
terial species from the placenta.

Blood for a serum sample was obtained from the dog
2 months after abortion and was tested for antibodies
against *L. infantum* by use of an indirect fluorescent anti-
body test (IFAT). Serum was examined at doubling dilu-
tions beginning at 1:25 in phosphate-buffered saline
(PBS) solution and titrated. Canine sera with known pos-
tive and negative results were included in each test as
controls. Approximately 50,000 *L. infantum* Virginia Tech-
1 promastigotes were placed in each well of a 12-well
IFAT slide, affixed with acetone, and stored at –20°C.
Serum (30 µL) was incubated on the slide for 30 minutes
at 22°C in a humidified box and washed twice for 5 min-
utes in PBS solution. Anti-dog IgG fluorescent conjugate\(^{c}\) at a dilution of 1:10 in PBS–Evan blue solution was added
to all wells and incubated for 30 minutes in a humidified
box. Slides were washed twice for 5 minutes in PBS solu-
tion and mounted in 90% glycerin-PBS solution. Slides
were viewed with an epifluorescent microscope.

Serum was also tested for K39 antigen, which is an
amastigote protein specific to *Leishmania* spp that
affects visceral organs and does not cross-react with
*Trypanosoma cruzi*.\(^{4}\) Anti-recombinant K39 (rK39) antibodies were evaluated by use of a qualitative commercial rK39 dipstick immunoassay\(^{d}\) according to the
manufacturer's test procedure.

Severe lesions were evident in the placenta and con-
sisted of necrosis of trophoblasts and infiltrations of
mixed leukocytes. Placental trophoblasts were heavily
parasitized with protozoa (Figure 1). The protozoan

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**Figure 1**—Photomicrographs of *Leishmania* spp amastigotes in sections of placenta of a naturally infected dog. Notice numerous amastigotes (arrows) in trophoblasts, including those with a visible kinetoplast (arrowheads). A—H&E stain; bar = 37 µm. B—H&E stain; bar = 15 µm. C—Toluidine blue stain; bar = 15 µm. D—Immunohistochemical stain; bar = 37 µm.
structures were unicellular; details were not clear, but in appropriately cut sections, a rodlike structure (kinetoplast) and a nucleus were recognized. These structures were more evident in 1-µm-thick toluidine blue–stained sections. Transmission electron microscopy revealed groups of protozoa in trophoblasts. Although tissue was autolyzed, a nucleus and a kinetoplast were clearly evident in amastigotes (Figure 2). These protozoa reacted with anti–L. infantum antibodies and not with anti–T. gondii anti–N. caninum antibodies or preinoculation rabbit serum.

Protozoa were not identified in sections of internal fetal tissues; histopathologic changes consisted of moderate diffuse hepatic cellular fatty changes in the liver and mild diffuse pulmonary edema and congestion in the lungs. Evidence of infection with canine parvovirus, canine distemper virus, canine herpesvirus, or canine coronavirus was not found. The IFAT titer in a serum sample was 1:100, and serum yielded positive results with the rK39 immunoassay.

Leishmania spp are flagellated protozoan parasites that are transmitted by the bite of infected female sand flies. Sand flies become infected with Leishmania spp when they ingest amastigotes while feeding on an infected host. Inside the digestive tract of the fly, the amastigotes become promastigotes. The promastigotes migrate to the hypostome of the sand fly and are inoculated when the fly feeds. The promastigotes are ingested by macrophages, and the new host is infected. Leishmania spp can cause visceral, cutaneous, or mucocutaneous disease in humans and other animals. Members of the Leishmania donovani complex cause human and canine visceral leishmaniasis in parts of Europe, the Middle East, Asia, China, and the Americas. Leishmania infantum is the member of the L. donovani complex identified in dogs in the United States and most often found in the Mediterranean basin, China, and the Middle East. Dogs are important reservoirs of human visceral leishmaniasis, and dog ownership is considered a risk factor for human visceral leishmaniasis in most areas of endemicity.

Until early 2000, canine visceral leishmaniasis was thought by most public health officials and veterinarians to be an unimportant disease in the United States. Most reported cases were in dogs that had originated in or traveled to areas where leishmaniasis is endemic. The recognition that L. infantum was actually endemic in Foxhounds in the United States in 1999 changed this thinking, and now, there is concern that the disease may make its way into the human population. Cases of leishmaniasis in dogs in the United States with no history of foreign travel have been reported.

Transmission of canine leishmaniasis is of scientific and public health concern because sand fly species proven to transmit L. infantum are not enzootic in the United States. Evidence indicates that transmission of Leishmania spp may also occur through blood or other bodily secretions. Transmission by blood transfusion and by packed RBC transfusion has recently been detected in dogs in North America that have received blood from donor Foxhounds. Congenital transmission of visceral leishmaniasis has been reported in humans, but this mode of transmission is uncertain in dogs. In a well-designed and controlled study of congenital transmission of leishmaniasis in dogs from Brazil, no evidence of congenital transmission was found in 63 puppies from 18 naturally infected dogs.

The diagnosis of leishmaniasis in the dog reported here was based on detecting serum antibodies against L. infantum, antibodies against rK39 in the serum, and Leishmania spp amastigotes in placental trophoblasts. The dog had an IFAT titer of 1:100 2 months after abortion occurred. An IFAT titer of 1:64 is considered diagnostic of Leishmania exposure, and IFAT antibodies were found in 2% of 12,000 Foxhounds. Organisms in placenta had morphologic characteristics of Leishmania or Trypanosoma amastigotes in that they possessed a kinetoplast. Positive results of the serum immunoassay for anti-rK39 antibodies ruled out T. cruzi because antibodies against T. cruzi do not cross-react in this test.

Protozoa are not a recognized cause of abortion in dogs. Although both T. gondii and N. caninum can cause neonatal death in dogs and neosporosis-induced abortion has been documented experimentally, there are no reports of T. gondii or N. caninum as a cause of naturally occurring abortion in dogs. The large numbers of characteristic organisms and associated lesions suggested the protozoal etiology of the abortion in the dog reported here, although Leishmania organisms were not seen in fetal tissues. Abortion related to canine leishmaniasis has been suggested in a dog from Italy. To our knowledge, this is the first report of leishmaniasis-associated placentalitis in a dog in the United States.


b. Dako, Carpinteria, Calif.

c. Kirkegaard & Perry Laboratories, Gaithersburg, Md.


References