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SURVEY OF ANTIBODIES TO *LEISHMANIA* SPP. IN WILD CANIDS FROM PENNSYLVANIA AND TENNESSEE

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Abstract: Visceral leishmaniasis (VL) is a zoonosis with worldwide distribution. Infections with the *Leishmania donovani* complex, including *Leishmania infantum*, cause the VL. Domestic dogs are the most important reservoir host for human VL, and wild canids are also susceptible. In the United States, infections with *L. infantum* are common in the foxhound dog breed. Little information is available regarding *L. infantum* in wild canids in the United States. Sera from 11 foxes and 256 coyotes originating in Pennsylvania and Tennessee (USA) were tested for antibodies to visceralizing *Leishmania* spp. with rapid immunochromatographic dipstick assays, which utilize recombinant antigen K39. Anti-*Leishmania* spp. antibodies were found in 5 of 267 (1.9%) of wild canids from Pennsylvania, including four coyotes and one red fox. These results suggest that wild canids are exposed to *Leishmania* spp. at a low level in the United States.

Key words: Antibody, coyote, fox, *Leishmania*, parasite.

BRIEF COMMUNICATION

Leishmaniasis is a potentially fatal sand fly-vector zoonosis caused by infection with protozoan parasites in the genus *Leishmania*. Visceral leishmaniasis (VL) results from infection with parasites in the *Leishmania donovani* complex, which includes *L. infantum*. Domestic dogs are the most important reservoir host of *L. infantum* for human infections. In the United States, leishmaniasis caused by *L. infantum* is a well-established disease in the foxhound dog breed, but the transmission mechanism of *L. infantum*

among foxhounds in the United States is unclear.¹⁰

In addition to domestic dogs, wild canids can serve as hosts for *L. infantum*. Infected domestic dogs may serve as the interface between wild and domestic cycles of VL by crossing into sylvatic environments and exposing wildlife to parasites.⁶ Little is known regarding *L. infantum* infections among wild canine species in the United States. Only a single report of antibodies to *Leishmania* in a United States wild canid has been recorded in a gray fox (*Urocyon cinereoargenteus*) from North Carolina.⁸ This study was conducted to determine the seroprevalence of *Leishmania* spp. among coyote and fox populations from Pennsylvania and Tennessee.

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Serum samples were collected from 267 wild canids from Pennsylvania and Tennessee (USA). Sera were taken from 16 trapper-collected coyotes from western Tennessee and from 240 coyotes (*Canis latrans*) and 11 red foxes (*Vulpes vulpes*) in Pennsylvania. Wild canine sera were collected throughout Pennsylvania by hunter harvest or by USDA-APHIS-Wildlife Services in order to settle wildlife damage problems. Serum samples were collected within 48 hr of death and frozen until tested. All samples were obtained legally.

Wild canine sera were tested for antibodies to visceralizing *Leishmania* spp. with the qualitative immunochromatographic (ICT) dipstick test. The ICT assay is commercially available for domestic dogs and previous studies have shown that they may also be used for wild canids.^{8,9} The ICT is based on recombinant antigen K39, which is an immunodominant amastigote protein specific for

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visceralizing *Leishmania* species. It does not cross react with *Trypanosoma cruzi*.³ The ICT is formatted as a proprietary gold mixture in a dipstick format. Canine sera were tested with the ICT using the manufacturer's directions (Kalazar Detect™ Canine Rapid Test, InBios International Ltd., Seattle, Washington, USA). For each test, 20 µl of thawed serum was used and test results were recorded after 10 min. A positive test demonstrated 2 red or pink bands, which represented a control line, and a second band in the test field. A negative test was indicated by only a single red control band.

Positive *Leishmania* spp. ICT results were found in 5 (1.9%) of 267 samples by ICT. All 5 antibody-positive wild canids were from Pennsylvania. *Leishmania* spp. antibodies were detected in 4 (1.7%) of 240 coyotes and 1 (9.1%) of 11 red fox from Pennsylvania. Antibodies to *Leishmania* spp. were not found in coyotes from Tennessee.

Leishmania spp. is an endemic zoonotic parasite in North America.^{4,6,9,10} *Leishmania infantum* is a well-recognized pathogen in the United States foxhound population.^{6,10} Domestic dogs are traditionally the primary reservoir host for human cases of sand fly-transmitted VL. Foxhound leishmaniasis in the United States, however, does not appear to be vectored by sand flies and vertical transmission may play a primary role in transmission.^{2,6}

Little information is available regarding the role of wild canine species as potential hosts of *L. infantum* in North America. Antibodies to *Leishmania* spp. were found previously using the *Leishmania* ICT in a single gray fox from North Carolina.¹⁰ The Centers for Disease Control and Prevention (CDC) conducted a serologic survey of 291 wild canids from the southeastern region of the United States and found no evidence of *Leishmania*.⁴ In a study conducted by the CDC, the immunofluorescent antibody test (IFAT) was used to screen wild canine sera and there was evidence of cross-reactivity to the related parasite, *Trypanosoma cruzi*, which is also endemic in the United States. Cross reaction to *T. cruzi* is an intrinsic limitation with the *Leishmania* IFAT, but the ICT used in the present study does not cross-react with *T. cruzi* antibodies.^{3,10}

The role of wild canine species as reservoir hosts for zoonotic leishmaniasis is unclear. The crab-eating fox (*Cerdocyon thous*) is well known for its high prevalence rate of *L. infantum* in Brazil and *Leishmania* spp. have been isolated from red foxes in Egypt and Portugal.^{1,5,7} The competence of infected wild canids as true reservoir hosts of

VL is not known.⁷ No confirmed cases of *L. infantum* infections have been reported in a wild canid from the United States. In the absence of sand fly transmission, the source of *Leishmania* exposure to wild canids is currently unknown. The role of infected coyotes and foxes as reservoir hosts for *Leishmania* spp. is probably minimal compared to other reservoirs, such as foxhounds.

This is the first report of antibodies to *Leishmania* spp. in a red fox and coyotes from the United States. Antibodies were detected in wild canids from Pennsylvania, but they were not found in any coyotes from Tennessee. Results from this study indicate that anti-*Leishmania* antibody levels are low which is in agreement with previous findings in wild canids.^{4,9,10} The ICT used in the present work is sold commercially for domestic dogs and results from this study indicate that they are a useful tool in screening coyotes and foxes for *Leishmania* spp.

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