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## Survey of Antibodies to *Trypanosoma cruzi* and *Leishmania* spp. in Gray and Red Fox Populations From North Carolina and Virginia

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**ABSTRACT:** American trypanosomiasis and leishmaniasis are caused by related hemoflagellate parasites, *Trypanosoma cruzi* and *Leishmania* spp., which share several common host species. Both zoonotic protozoans are endemic in the United States. Canines, including domestic and wild canids, are reservoir hosts for human infections with *T. cruzi* and *Leishmania* spp. The present study examined the seroprevalence of *T. cruzi* and *Leishmania* spp. in wild canids from North Carolina and Virginia. Wild canine species tested in this work included 49 gray foxes (*Urocyon cinereoargenteus*) and 5 red foxes (*Vulpes vulpes*). Overall, sera samples from 54 foxes (North Carolina = 43; Virginia = 11) were tested by immunochromatographic strip assays (ICT). Antibodies to *T. cruzi* were found in 4 (9%) gray foxes from North Carolina and 2 (18%) gray foxes from Virginia. Antibodies to *Leishmania* spp. were detected in 1 (2%) gray fox from North Carolina. Our results indicate that wild canids are exposed more frequently to *T. cruzi* in North Carolina than *Leishmania* spp. and only *T. cruzi* in Virginia.

*Trypanosoma cruzi* and *Leishmania* spp. are insect-transmitted zoonotic hemoflagellates that are endemic in the United States. These closely related protozoan parasites can be fatal in infected people and dogs. In the United States, leishmaniasis caused by *Leishmania infantum* is an important disease in the foxhound breed. *Trypanosoma cruzi* is the etiological agent of Chagas' disease in mammals and *T. cruzi*-infected foxhounds have been reported in North America (Duprey et al., 2006).

In the United States, *T. cruzi* is routinely diagnosed in wild mammals (Brown et al., 2010). The sylvatic life cycle involves *T. cruzi* transmission between triatomine bugs and wildlife reservoir hosts. It is well documented that *T. cruzi* and *Leishmania* spp. infect domestic dogs throughout much of the United States (Rosypal et al., 2003; Duprey et al., 2006). Few studies, however, have examined the seroprevalence of *T. cruzi* and *Leishmania* spp. in wild canids in the United States; therefore, the present study was conducted to determine the prevalence of antibodies to *Leishmania* spp. and *T. cruzi* in wild canine populations from North Carolina and Virginia.

For the present study, 54 wild canine samples were collected from North Carolina and Virginia. Sera samples from 49 gray foxes (*Urocyon cinereoargenteus*) and 5 red foxes (*Vulpes vulpes*) were obtained in North Carolina and Virginia as part of a predator removal study. Foxes were live-trapped in 3 counties in North Carolina (Hyde, Tyrell, and Wilson) and 1 county in Virginia (Amelia) in February–April 2000. Study sites in Hyde County (35°42'N, 76°05'W) and Tyrell County (35°50'N, 76°14'W) were located in the lower coastal plain of North Carolina and were characterized by farm fields, surrounding timber, and sparse human populations. The study site in Wilson County (35°42'N, 77°53'W) is located in North Carolina's upper coastal plain and includes agricultural fields and surrounding timber. While more populated than areas on the lower coastal plain, it is still mostly rural. Amelia County (37°47'N, 77°92'W) is centrally located in Virginia's Piedmont region and is 73% forested.

Animals were anesthetized with telazol (Fort Dodge Animal Health, Fort Dodge, Iowa) (Stoskopf et al., 1999), blood samples were taken, and the animals were killed with sodium pentobarbital (Beuthanasia-D Special, Schering Plough Animal Health, Summit, New Jersey). Serum was extracted and frozen at –70 C.

Qualitative antibody testing was conducted with commercial canine immunochromatographic (ICT) dipstick assays for *T. cruzi* and visceralizing *Leishmania* spp. Canine ICT assays developed for diagnostic use in domestic dogs are commercially available. A previous study showed that they perform as well as the generally accepted "gold standard," indirect immunofluorescent antibody test (IFAT), when using wild canine sera (Rosypal et al., 2007). The ICT tests are a proprietary gold mix based on recombinant antigens and have been developed into a dipstick format. Previous reports have demonstrated their superior performance over traditional serological screening tests based on crude antigens or whole organisms (Houghton et al., 2000; Scalone et al., 2002). The canine *T. cruzi* ICT (*Trypanosoma* Detect™ MRA Rapid Test; Inbios International Ltd., Seattle, Washington) is based on multi-epitope recombinant antigens and was used for fox sera to detect anti-*T. cruzi* antibodies. Fox sera were also tested for antibodies to recombinant K39 (rK39) (Kalazar Detect™ Canine Rapid Test, InBios International Ltd., Seattle, Washington), which is an amastigote protein specific to visceral *Leishmania* spp. that does not cross-react with antibodies to *T. cruzi* (Burns et al., 1993).

Serology was performed by ICT according to the manufacturer's test procedure. Briefly, wild canine serum (20 µl) was pipetted onto the test strip. The dipstick then was placed in a well of a 96-well round-bottom tissue culture plate, and aliquots of manufacturer-provided chase buffer (100 µl to 150 µl) were introduced into the well. Test results were read after 10 min. According to the test procedure, after 10 min the appearance of a red control line and a second line in the test field was interpreted as positive. The presence of red band only on the control line indicated a negative result.

Positive *T. cruzi* ICT results were detected in the sera of 6 (11%) of the 54 wild canids from North Carolina and Virginia. Four gray foxes from Wilson and Tyrell Counties in North Carolina and 2 gray foxes from Amelia County, Virginia, were antibody positive. *Leishmania* spp. antibodies were found by ICT in serum from 1 (2%) male gray fox, from Wilson County, North Carolina. This *Leishmania* spp. seropositive gray fox did not have detectable antibodies to *T. cruzi*. None of the red foxes from North Carolina and Virginia presented evidence of antibodies to either *T. cruzi* or *Leishmania* spp.

*Leishmania* spp. and *T. cruzi* are endemic zoonotic parasites in canines in the United States and Canada (Rosypal et al., 2003; Duprey et al., 2006; Rosypal et al., 2007). Canine leishmaniasis caused by *Leishmania infantum* is well established in the North American foxhound population, and *Leishmania* spp. and *T. cruzi*-infected foxhounds have been detected throughout most of the eastern half of the United States and as far north as Ontario, Canada (Duprey et al., 2006). In a serological survey conducted by the Centers for Disease Control and Prevention, there was no evidence of either confirmed or probable cases of *Leishmania* spp. in 291 wild canids from the southeastern United States (Duprey et al., 2006). In the same study, 2 of 291 (0.7%) wild canids had antibodies to *T. cruzi* (Duprey et al., 2006). There was evidence of cross-reactions to *Leishmania* spp. by IFAT because these canids were also positive for *T. cruzi* antibodies (Duprey et al., 2006). The low *Leishmania* spp. antibody responses in these 2 wild canids were most likely false positives due to cross-reactivity in the IFAT to *T. cruzi* antibodies (Duprey et al., 2006), which is an inherent limitation of the *Leishmania* spp. IFAT (Rosypal et al., 2003). The *Leishmania* spp. ICT used in the present study does not cross-react with *T. cruzi* (Burns et al., 1993). To our knowledge, this is the first report of antibodies to *Leishmania* spp. in wild canids from the United States.

In North America, *T. cruzi* is found in various mammalian wildlife hosts (John and Hoppe, 1986; Pung et al., 1995; Brown et al., 2010). Raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) are the most commonly described reservoirs (Brown et al., 2010). Wild mammals are routinely exposed to *T. cruzi* in the southern United States (reviewed by Diaz, 2007; Brown et al., 2010), and *T. cruzi*-positive foxhounds have been reported as far north as Ontario, Canada. A previous study reported the seroprevalence of antibodies to *T. cruzi* to be 12.8% among 156 coyotes (Burkholder et al., 1980) from Texas evaluated by the indirect hemagglutination test. In another study from Texas, 14.2% of 134 coyotes were found to be positive by IFAT (Grögl et al., 1984). In South Carolina, 8% of 26 gray foxes were positive by IFAT and ICT (Rosypal et al., 2007). A recent IFAT study detected *T. cruzi* antibodies in 3.8% of 26 coyotes from Virginia and 4.4% of 23 coyotes from Georgia (Brown et al., 2010).

The present study reveals that foxes are exposed to *T. cruzi* in North Carolina and in Virginia. In the United States, the prevalence of *T. cruzi* is highest in raccoons and opossums, with numbers ranging from 1.5% to 63% (reviewed by Brown et al., 2010), but infection varies by geography and host species. The *T. cruzi* antibody levels from North Carolina described here are similar to levels in wild canids from Texas and South Carolina (Burkholder et al., 1980; Grögl et al., 1984; Rosypal et al., 2007). Our data suggest that wild canids are less susceptible or they have a lower exposure to *T. cruzi* than raccoons and opossums. The role of wild canids as reservoir hosts for *T. cruzi* is probably minimal compared to other reservoir host species. This is the first report of antibodies to *Leishmania* spp. in a gray fox from the United States, but the low levels are in agreement with previous findings in wild canids (Duprey et al., 2006; Rosypal et al., 2007). The ICT assays used in the present work are commercially available for domestic canids, and our results indicate that they are a useful tool in screening wild canids for *T. cruzi* and *Leishmania* spp.

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