Prevalence of Antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in Wild Canids From South Carolina

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Prevalence of Antibodies to *Leishmania infantum* and *Trypanosoma cruzi* in Wild Canids From South Carolina

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ABSTRACT: Wild canids are reservoir hosts for *Leishmania infantum* and *Trypanosoma cruzi*. The present study examined the prevalence of antibodies to these zoonotic parasites in a population of wild canids from a nonagricultural setting in South Carolina. Sera from 26 gray foxes (*Urocyon cinereoargenteus*) and 2 coyotes (*Canis latrans*) were examined for antibodies to *L. infantum* and *T. cruzi* using the indirect immunofluorescent antibody test and commercially available parasite-specific immunochromatigraphic strip assays. Antibodies to *L. infantum* were not detected by either assay in gray foxes or coyotes. Two (8%) of 26 gray foxes were positive in both the *T. cruzi* immunofluorescent antibody and strip assays. Antibodies to *T. cruzi* were not detected in coyotes. Results from this study indicate that wild canids are exposed to *T. cruzi*, but not *L. infantum*. in this geographic region.

Leishmania infantum and *Trypanosoma cruzi* are zoonotic parasites and both are endemic in the United States. Leishmaniasis and Chagas' disease caused by infection with *L. infantum* and *T. cruzi*, respectively, are potentially fatal diseases in both dogs and humans. Canine leishmaniasis caused by *L. infantum* is recognized as an important disease in the North American foxhound population, and *T. cruzi*-infected foxhounds also have been described (Duprey et al., 2006).

A sylvatic cycle of *T. cruzi* exists that involves parasite transmission between reduviid bugs and wildlife reservoir hosts. Little data are available, however, regarding *L. infantum* and *T. cruzi* infections in wild canids in the United States. The present study was conducted to determine the seroprevalence of antibodies to *L. infantum* and *T. cruzi* in a population of gray foxes (*Urocyon cinereoargenteus*) and coyotes (*Canis latrans*) from South Carolina.

Sera from 26 gray foxes (Lindsay et al., 2001) and 2 coyotes from South Carolina were examined for antibodies to *L. infantum* and *T. cruzi*. The wild canids examined in the present study originated from a nonagricultural setting in Aiken and Barnwell counties on the Department of Energy's Savannah River site. Other wildlife species are present in the study area, including white-tailed deer (*Odocoileus virginianus*), and they are hunted with domestic dogs only during hunting season; otherwise, domestic dogs are not allowed in the study location. The animals were collected alive in padded leg-hold traps and sedated using intramuscular injections of ketamine and xylazine. Animals were bled while unconscious. After venipuncture, animals were humanely killed for other studies on the population of wild canids in this study site. The age of each fox was determined using tooth cementum analysis of an extracted lower premolar. Ages of coyotes were not available. Sera were

examined by the quantitative indirect fluorescent antibody test (IFAT) and commercially available immunochromatographic dipstick assays for qualitative antibody detection.

The IFAT was used to examine sera for IgG antibodies to *T. cruzi* and *L. infantum* as described previously (Dubey et al., 2005; Rosypal et al., 2005). Briefly, sera were initially screened at a 1:25 dilution in phosphate-buffered saline (PBS). Positive samples were examined at doubling dilutions and titrated to a final dilution of 1:12,800. In the *T. cruzi* IFAT, Brazil strain amastigotes and trypomastigotes were washed in PBS, and approximately 40,000 parasites per well were air-dried on 12-well microscope slides. Parasite antigen was affixed on IFAT slides with acetone. Sera (30 μl/well) were diluted to 1:25 in PBS and incubated on the slides for 30 min at room temperature in a humidified box. Slides were washed 2 times for 5 min in PBS. The secondary antibody used in the present study was fluorescein isothiocyanate-conjugated goat anti-dog IgG (H+L) (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland). Fluorescent conjugate (30 μl/well) at a dilution of 1:10 in PBS-Evans blue solution was incubated on the slides for 30 min at room temperature in a humidified box. Slides were washed twice for 5 min in PBS, mounted in Fluoromount-G (Southern Biotechnology Associates Inc., Birmingham, Alabama), and covered with coverslips (24 × 60 mm). Slides were viewed with an Olympus BX60 epifluorescent microscope equipped with differential contrast optics. The procedure was the same as described above for the *Leishmania* IFAT, except 50,000 promastigotes of *L. infantum* (ATCC PRA-149) (canine isolate from a naturally infected Virginia foxhound; Rosypal, Troy, Zajac et al., 2003) per well were used as antigen. Canine control sera were used on each IFAT slide. Sera from dogs with proven *T. cruzi* and *L. infantum* infections were used as positive controls for respective IFAT tests. Negative control sera used in both IFATs was obtained from a dog proven to be uninfected by both culture and serology.

Sera also were tested qualitatively by commercially available canine immunochromatographic dipstick assays according to the manufacturer's test procedure. The tests are based on recombinant antigens that previous reports have demonstrated superior performance over traditional serological screening tests based on crude antigens or whole organisms (Houghton et al., 2000; Scalone et al., 2002). Canine sera were tested for anti-recombinant K39 (rK39) (Kalazar Detect™ Canine Rapid Test; InBios International Ltd., Seattle, Washington), which is an amastigote protein specific to visceralizing *Leishmania* spp., and it does not crossreact *T. cruzi* (Burns et al., 1993). Anti-*T. cruzi* antibodies were evaluated by a dipstick assay (Trypanosoma Detect™ MRA Rapid Test; InBios International Ltd.) based on multiepitope recombinant antigens. The IFAT was considered the ''gold standard'' for comparisons of sensitivity and specificity, because it has been shown to have a high sensitivity for detecting antibodies to *T. cruzi* in wildlife (Yabsley et al., 2001).

Positive *T. cruzi* IFAT and *T. cruzi* dipstick assay results were found in 2 (8%) of 26 gray foxes. One 5-yr-old male fox from Aiken County and 1 7-yr-old female fox from Barnwell County were both IFAT positive at a final titer of 1:25. None of the coyotes had antibodies to *T. cruzi* by either IFAT or dipstick tests. None of the wild canids tested in the present study had evidence of antibodies to *L. infantum* by either serological assay. The sensitivity and specificity of the dipstick assays were 100% compared with IFAT as the gold standard.

Leishmania infantum and *T. cruzi* are endemic parasites in the United States and Canada (Rosypal, Zajac, and Lindsay, 2003; Rosypal et al., 2005; Duprey et al., 2006). Autochthonous canine leishmaniasis caused by *L. infantum* is well recognized in the North American foxhound population, and *Leishmania* sp.-infected and *T. cruzi*-infected foxhounds have been detected in South Carolina (Duprey et al., 2006). The lack of antibodies to *L. infantum* in wild canids examined in this study is in agreement with previous findings (Duprey et al., 2006). Serological surveys conducted by the Centers for Disease Control and Prevention indicated that 2 of 291 (0.7%) wild canid samples collected in the southeastern United States had antibodies to *T. cruzi*, although both the exact collection location and wild canine species were unclear (Duprey et al., 2006).

Trypanosoma cruzi infection has been described in a variety of wild animals in the southeastern United States (John and Hoppe, 1986; Pung et al., 1995). Seropositive raccoons have been reported from both urban (Hancock et al., 2005) and nonurban (Yabsley et al., 2002a, 2002b) areas in the United States, including South Carolina. In contrast to our study, 19 of 134 (14.2%) seropositive coyotes were detected in Texas (Grögl et al., 1984), although only 2 coyotes were tested in the present work. McKeever et al. (1958) isolated *T. cruzi*-like parasites from 2 of 118 (1.7%) gray foxes collected in southwestern Georgia and northwestern Florida. Our report, however, is apparently the first to document antibodies to *T. cruzi* in gray foxes from South Carolina.

The immunochromatigraphic strip assays used in this study are commercially available for domestic dogs, but they have not been validated using wild canine serum. The strip tests are qualitative, and the intensity of the chromagen in the test region varies depends on the concentration of antibodies present in the sample. According to the manufacturer, a faint line should be considered a positive result. The positive *T. cruzi* strip assays in 2 gray foxes in the current study had positive IFAT results at a concentration of 1:25. We compared results of the dipstick assays to IFAT, and we found 100% sensitivity and specificity compared with the gold standard. To our knowledge, this is the first serological study using the *Leishamania* sp. and *T. cruzi* dipstick assays in wild canids. Dipstick assays are simple, rapid tests that are easier to perform than IFAT. Results from this work indicate that these tests warrant further study with additional gray fox samples and possibly other wild canine species to validate their usefulness as a serological screening tool in wild canids.

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Distribution Pattern of Phthirapterans Infesting Certain Common Indian Birds

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ABSTRACT: The prevalence and frequency distribution patterns of 10 phthirapteran species infesting house sparrows, Indian parakeets, common mynas, and white breasted kingfishers were recorded in the district of Rampur, India, during 2004–05. The sample mean abundances, mean intensities, range of infestations, variance to mean ratios, values of the exponent of the negative binomial distribution, and the indices of discrepancy were also computed. Frequency distribution patterns of all phthirapteran species were skewed, but the observed frequencies did not correspond to the negative binomial distribution. Thus, adult–nymph ratios varied in different species from 1:0.53 to 1:1.25. Sex ratios of different phthirapteran species ranged from 1:1.10 to 1:1.65 and were female biased.

Certain workers have provided useful information on the prevalence and frequency distribution pattern of Phthiraptera on selected avian hosts. Work done so far on the subject has been reviewed from time to time (Marshall, 1981; Price and Graham, 1997; Price et al., 2003). The patterns of abundance of different lice on their avian hosts have been further discussed by Rekasi et al. (1997), Rozsa (1997), and Reiczigel et al. (2005). Here, we report information on the prevalence and frequency distribution patterns of 10 different phthirapteran species parasitizing some common Indian birds: house sparrow (*Passer domesticus*), Indian parakeet (*Psittacula eupatria*), common myna (*Acridotheres tristis*), and white-breasted kingfisher (*Halcyon smyrnensis*).

All birds were captured live (with mist nets) in the district of Rampur (U.P.), India, during 2004 and 2005. After tying the legs, each bird was thoroughly searched for the presence of lice by visual examination. Infested birds were then subjected to delousing by fumigation (modified ''Fair-Isle'' method). The efficacy of different methods for delousing infested birds has been discussed elsewhere (Clayton and Drown, 2001). Head and body feathers were further examined using a stereozoom trinocular microscope to remove the remaining lice. The entire louse load was placed in 70% alcohol and separated according to species, age, and gender. The prevalence (%), mean (X), and variance (s^2) were calculated. The exponent (k) of negative binomial distribution and index of discrepancy (D) (Rozsa et al., 2000) were generated, and the goodness of fit between observed and expected frequency distribution was determined by chi-square tests.

Three phthirapteran species, *Brueelia subtilis* (Nitzsch, 1874), *Echinophilopterus chapini* (Ewing, 1927), and *Myrsidea quadrifasciata* (Piaget, 1880), were recovered from 100 house sparrows (Table I). Frequency distribution patterns of the 3 species were skewed, but were not

TABLE I. Summary of distribution patterns of phthirapterans infesting of house sparrows, Indian parakeets, common myna, and white-breasted kingfishers in the district of Rampur, India, during 2004 and 2005.

Host	Sample size	Louse species	Prevalence (%)	Sample mean abundance	Range of infestation	Mean intensity (X)	Variance to mean ratio (s^2)	Exponent of negative binomial (k)	Index of discrepancy (D)
House sparrow	100	*Brueelia subtilis*	31.0	4.1	1–41	13.3	16.0	0.11	0.79
	100	*Echinophilopterus chapini*	14.0	1.1	2–21	7.6	10.6	0.05	0.90
	100	*Myrsidea quadrifasciata*	20.0	1.5	2–28	9.7	12.8	0.07	0.87
Indian parakeet	100	*Neopsittaconirmus elbeli*	34.0	7.4	2–46	21.8	21.2	0.11	0.76
	100	*Echinophilopterus chapini*	17.0	13.8	1–28	13.8	16.6	0.05	0.88
Common myna	100	*Myrsidea invadens*	31.0	5.1	2–63	16.3	32.1	0.10	0.86
	100	*Menacanthus eurysternus*	13.0	2.3	2–32	17.5	20.8	0.03	0.90
	100	*Brueelia chayanh*	24.0	6.9	3–82	28.9	41.9	0.06	0.86
	100	*Sturnidoecus bannoo*	42.0	15.6	3–106	37.07	50.3	0.12	0.78
White-breasted kingfisher	30	*Meropoecus* sp.	40.0	7.1	6–44	17.75	20.09	0.15	0.71