Surveillance for Antibodies to *Leishmania* spp. in Dogs from Sri Lanka

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Published By: American Society of Parasitologists

DOI: [http://dx.doi.org/10.1645/GE-2288](http://dx.doi.org/10.1645/GE-2288)

ABSTRACT: The global distribution of leishmaniasis is rapidly expanding into new geographic regions. Dogs are the primary reservoir hosts for human visceral leishmaniasis caused by infection with *Leishmania infantum*. Natural infections with other *Leishmania* spp. can occur in dogs, but their role as reservoir hosts for other species of *Leishmania* is uncertain. *Leishmania donovani* is traditionally considered a visceralizing anthropopnotic species; however, cutaneous leishmaniasis caused by *L. donovani* has been reported in Sri Lanka. In the present study, sera from 114 dogs in Sri Lanka were examined for antibodies to visceralizing *Leishmania* spp. Sera were tested by the canine immunochromatographic strip assay based on recombinant K39 antigen. Antibody testing was performed according to the manufacturer's test procedure (Kalazaz Detect Canine Rapid Test, In BIOS International Ltd., Seattle, Washington). The ICT assay is based on recombinant antigens and has been developed into a dipstick format. The tests are based on recombinant antigens for which previous reports have demonstrated superior performance over traditional serological screening tests based on crude antigens or whole organisms (Miall et al., 2002). Canine sera were tested for antibodies to recombinant K39 (K39), which is an amastigote protein specific to visceralizing *Leishmania* spp. (Burns et al., 1993). The ICT detects antibodies to *L. infantum* and *L. donovani*, which are both parasites in the *L. donovani* complex.

Of the 114 Sri Lanka dogs assayed, 1 (0.9%) had detectable antibodies to *Leishmania* sp. There are several serological methods used for detection of *Leishmania* sp. –specific antibodies, including the indirect fluorescent antibody test (IFAT), complement fixation, direct agglutination, enzyme-linked immunosorbent assay, and ICT dipstick tests (Rosypal et al., 2003; Lira et al., 2006). The IFAT is the most commonly used of all serological assays and represents the “gold standard” with which other serological tests are compared (Rosypal et al., 2003). Serological tests are not 100% sensitive and specific; however, several studies using the recombinant antigens in the ICT demonstrated high specificity compared to traditional serological assays (Burns et al., 1993; Rosypal et al., 2005, 2007). In addition, the ICT assay used in the present study is now considered to be the best available test for field diagnosis of VL in remote geographic regions (Chappuis et al., 2007).

Cutaneous leishmaniasis caused by *L. donovani* infection can occasionally occur, as in reports from Sri Lanka (Navaratna et al., 2007), making further investigation necessary for Sri Lankan *L. donovani*. Multilocus enzyme electrophoresis characterized cutaneous *L. donovani* strains from Sri Lanka as zymodeme MON-37 (Karunaweera et al., 2003), which differs from India, where VL is predominantly caused by *L. donovani* zymodeme MON-2. DNA sequencing and microsatellite analyses demonstrated that Indian and Sri Lankan *L. donovani* strains are genetically similar, but highlighted the important biological differences between geographically close parasites (Srivardana et al., 2007).

There are at least 15 *Leishmania* spp. that are pathogenic for humans and 13 are well-recognized zoonotic parasites (Gramiccia and Gradoni, 2005). Only 2 species, *L. tropica* and *L. donovani*, are traditionally considered to be anthropopnotic organisms. Moreover, there is disagreement over the transmission dynamics of these 2 species since natural infections in animal reservoir hosts have been documented in endemic areas (reviewed by Gramiccia and Gradoni, 2005). In Asia and other parts of the world, dogs are primary reservoir hosts of *L. infantum*, although canine infections are possible with *L. donovani* (Dereure et al., 2003), the causative agent of human VL in India. Indeed, biological differences among geographically distinct isolates of a single *Leishmania* sp. and
zymodemes have been described (Cupolillo et al., 2003; Rosypal et al., 2005). We investigated the prevalence of antibodies to visceralizing Leishmania sp. parasites in a canine population from Sri Lanka. Results from our study support previous work, suggesting that leishmaniasis may be an emerging zoonosis in Sri Lanka. Although the presence of Leishmania sp. antibodies in a single dog is insufficient to confirm a canine reservoir, further studies should be conducted to elucidate the role of dogs in Sri Lankan leishmaniasis.

This work was supported by a grant from the Bill and Melinda Gates Foundation to R.R.T. The contribution of S. T. and C. K. was funded by a mini-grant to A.C.R. from the Historically Black Universities and Colleges-Undergraduate Program (HBCU-UP) at Johnson C. Smith University.

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