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Author(s): A. C. Rosypal , S. Tripp , C. Kinlaw , S. Hailemariam , R. R. Tidwell , D. S. Lindsay , R. P. V. J. Rajapakse , C. Sreekumar , and J. P. Dubey
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Surveillance for Antibodies to *Leishmania* spp. in Dogs From Sri Lanka

A. C. Rosypal, S. Tripp, C. Kinlaw, S. Hailemariam*, R. R. Tidwell†, D. S. Lindsay‡, R. P. V. J. Rajapakse§, C. Sreekumar‖, and J. P. Dubey#,
Department of Natural Sciences and Mathematics, College of Science, Technology, Engineering and Mathematics, Johnson C. Smith University, 100 Beatties Ford Rd., Charlotte, North Carolina, 28216; *Department of Natural Sciences and Mathematics, College of Arts and Sciences, Shaw University, Raleigh, North Carolina, 27601; †School of Medicine, Department of Pathology and Laboratory Medicine, CB #7525, Room 805 Brinkhous-Bullitt Building, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 25799; ‡Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, Virginia 24061; §Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka; ‖Department of Parasitology, Madras Veterinary College, Tamil Naidu Veterinary and Animal Sciences University, Chennai-600007, India; #United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, Maryland 20705-2350. *e-mail: acrosypal@jcsu.edu*

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 anthroponotic 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 assays based on recombinant K39 antigen. Anti-*Leishmania* spp. antibodies were detectable in 1 of 114 (0.9%) dogs from Sri Lanka. Nonetheless, serological evidence suggests that leishmaniasis may be an emerging zoonosis in Sri Lanka.

Visceral leishmaniasis (VL) is a systemic sand fly–vectored disease caused by infection with protozoan parasites in the *Leishmania donovani* complex, which includes *Leishmania infantum*. Zoonotic VL, caused by infection with *L. infantum*, is endemic in the Americas, the Mediterranean basin, and parts of Asia, including China and the Middle East (Dereure et al., 2003). The domestic dog is the primary reservoir host for human cases of zoonotic VL. In the Indian subcontinent and East Africa, anthroponotic VL results from *L. donovani* (sensu stricto) transmission from human to insect vector to human. However, although *L. donovani* is generally considered a viscerotropic parasite, it has also been sporadically implicated in cases of cutaneous leishmaniasis in India and Sri Lanka (Karunaweera et al., 2003; Nawaratna et al., 2009; Sharma et al., 2009). Recently, in Sri Lanka, increasing numbers of cutaneous leishmaniasis caused by *L. donovani* infection have been reported and there is limited evidence suggesting that it may be a zoonotic form of the disease (Nawaratna et al., 2009). Leishmaniasis has been reported in regions of the world where it was not found previously (Rosypal et al., 2003; Shaw, 2007). Several factors influence the increased incidence of VL, including human migration, HIV co-infection, and climate change (Chappuis et al., 2007; Shaw, 2007).

Evolutionary history suggests that species of *Leishmania* have a zoonotic origin and that certain species, including *L. donovani*, have adapted to anthroponotic transmission cycles (Kerr, 2000; Kerr et al., 2000; Shaw, 2007). Anthroponotic VL is caused by infection with *L. donovani* and, moreover, dogs are generally not considered to be involved in the transmission cycle of this parasite to humans. Natural infection of dogs with *L. donovani* can, however, occur (Dereure et al., 2003), but the status of the domestic dog as a reservoir is unclear (Dantas-Torres, 2007). The purpose of the present study was to determine the prevalence of antibodies to visceralizing *Leishmania* parasites in canine populations from Sri Lanka.

In this investigation, 114 street dogs were captured in Sri Lanka and killed by overdose injection of sodium thiopentonel. Both male and female mixed-breed dogs of varying ages were included in the study group. Information on individual dogs was not available. Blood samples were collected at necropsy as part of prevalence studies for *Toxoplasma gondii* (Dubey et al., 2007); refrigerated serum was sent by air to the United States Department of Agriculture Animal Parasitic Diseases Laboratory, Beltsville, Maryland, in March and June 2006. Serum samples were stored at −20 C, and frozen sera were subsequently sent to the Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. Further serologic testing was conducted at Shaw University, Raleigh, North Carolina, and Johnson C. Smith University, Charlotte, North Carolina.

The commercially available immunochromatographic (ICT) dipstick assay was performed for qualitative detection of antibody to visceralizing *Leishmania* spp. Antibody testing was performed according to the manufacturer's test procedure (Kalazar Detect Canine Rapid Test, InBios 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 (Scalone et al., 2002). Canine sera were tested for antibodies to recombinant K39 (rK39), 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 anti-*Leishmania* sp. antibodies. 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 (Nawaratna 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 (Siriwardana 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 anthroponotic 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 infection is 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

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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.

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