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## Serological survey of *Leishmania infantum* and *Trypanosoma cruzi* in dogs from urban areas of Brazil and Colombia

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### Abstract

*Leishmania infantum* and *Trypanosoma cruzi* are zoonotic parasites that are endemic throughout many parts of Latin America. Infected dogs play an important role in transmission of both parasites to humans. A serological survey of *Leishmania* and *Trypanosoma* infection was conducted on 365 dogs from São Paulo, Brazil and Bogotá, Colombia, South America. Serum samples were examined by the indirect immunofluorescent antibody test (IFAT). Anti-*Leishmania* IgG antibodies were detected in 5 of 107 from Brazil (4.7 %) and in 4 of 258 dogs (1.6%) from Colombia. Titers ranged from 1:25 to 1:100. Anti-*T. cruzi* antibodies were not detected in any of the dogs from either Brazil or Colombia. The results show a low prevalence of anti-*Leishmania* antibodies and no antibodies against *T. cruzi* in these canine populations. Our study suggests that dogs play a limited role in the spread of *L. infantum* and *T. cruzi* in these urban areas of Brazil and Colombia.

### Keywords

*Leishmania infantum*; *Trypanosoma cruzi*; Dog; Antibody; Indirect immunofluorescent antibody test; Brazil; Colombia

### 1. Introduction

*Leishmania* and *Trypanosoma* spp. are closely related hemoflagellates that are endemic in Central and South America. Visceral leishmaniasis (VL) is a potentially fatal disease caused

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by infection with protozoan parasites in the *Leishmania donovani* complex, which includes *L. infantum* (syn. *L. chagasi*, Mauricio et al., 2000). In Latin America, vertebrate hosts become infected with *L. infantum* by infected blood feeding phlebotomine sand flies, primarily *Lutzomyia longipalpis* (Miles et al., 1999). *T. cruzi* is the etiologic agent of American trypanosomiasis, or Chagas' disease, and is vectored by hematophagous triatomine arthropods, including the domestic vectors, *Triatoma infestans* and *Rhodnius prolixus* (Dias et al., 2002). Both *L. infantum* and *T. cruzi* have been detected in a wide range of domestic and wild animals, including dogs, cats, and rodents. Domestic dogs are principal reservoir hosts for human infections with both parasites and they play an important role in the epidemiology of VL and Chagas' disease.

Every year, there are approximately 500,000 new cases of VL (Desjeux, 2001; Desjeux, 2004) which cause 59,000 human deaths annually (WHO, 2002) and incidence is increasing (Desjeux, 2001). It is estimated that 90% of all new cases of VL occur in five developing countries: India, Nepal, Bangladesh, Sudan, and Brazil (Desjeux, 2004). Dogs are well recognized as important reservoirs for human VL in endemic areas (Ashford et al., 1998) and dog ownership is considered a risk factor for human infections (Gavagni et al., 2002). In the state of São Paulo, the first case of canine VL was reported in 1998 from the municipality of Araçatuba and one year later the first human VL case was detected (Camargo Neves, 2004). In Brazil, a public health campaign targeting dogs is used to reduce the incidence of human VL by culling seropositive dogs (Ashford et al., 1998). The effectiveness of this intervention strategy, however, has long been debated (Dye, 1996; Tesh, 1995).

The World Health Organization (WHO) estimates 16–18 million people are infected with *T. cruzi* in Latin America with 100 million people at risk (WHO, 2007). International eradication programs including Southern Cone countries (Brazil, Paraguay, Uruguay, Argentina, Chile, and Bolivia) and Andean countries (Colombia, Chile, Bolivia, Ecuador, Peru, Venezuela) were initiated as early as 1991 in an effort to eliminate Chagas' disease (Kirchoff, 2006). Control strategies are primarily focused on vector elimination by spraying pyrethroid insecticides and prevention of disease transmission by blood transfusion (Dias et al., 2002). Infected dogs, however, are common domestic reservoir hosts and they are a risk factor as a source of *T. cruzi* infection for humans (Crisante et al., 2006; Gürtler et al., 2006).

Public health concerns associated with *L. infantum* and *T. cruzi* infections reveal the need for investigation of these infections in dogs that can be sources for subsequent parasite transmission to humans. American trypanosomiasis and VL are focal diseases and tremendous variability exists in their epidemiology (Diosque et al., 2004; Moncayo et al., 2006; Dantas-Torres et al., 2006a). Both parasitoses are common in rural areas (Gomes et al., 2007; Cohen et al., 2001), but increasing reports suggest disease urbanization (Moncayo et al., 2006; França-Silva et al., 2003). Information regarding prevalence and geographic distribution of canine infections is essential for developing and monitoring strategic control measures. The purpose of the present study was to determine the prevalence of antibodies to *L. infantum* and *T. cruzi* in two canine populations from São Paulo, Brazil and Bogotá, Colombia.

## 2. Materials and methods

### 2.1 Dogs and study area

Samples were obtained from 365 domestic dogs from Brazil and Colombia, South America. A total of 258 unclaimed dogs (6 months or older) were collected between February and May 2006 from Bogotá, Colombia. After efforts to place the dogs as pets failed, they were humanely euthanized by intravenous injection (Euthanex®, Invet, S.A. Bogotá, Colombia) by Centro Distrital de Zoonosis, Bogotá.

One-hundred seven unwanted dogs (1 year or older) were killed between December 2005 and April 2006 from São Paulo, Brazil. The dogs were unclaimed pets or they were caught by the Municipality of São Paulo. They were taken to the Center for Zoonosis, São Paulo where the dogs were euthanized by overdose injection of phenobarbital.

## 2.2 Collection of sera

Blood samples were collected at necropsy. Serum was separated by centrifugation, and refrigerated sera were originally sent by air to the United States Department of Agriculture Animal Parasitic Diseases Laboratory, Beltsville, MD as part of a *Toxoplasma gondii* prevalence and genetic characterization study (Dubey et al., 2007a,b). Serum samples were stored at  $-70^{\circ}\text{C}$  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, NC for serologic testing.

## 2.3 Serology

The indirect immunofluorescent antibody test (IFAT) was performed to detect anti-*Leishmania* and anti-*Trypanosoma* IgG antibodies in canine sera as previously described (Dubey et al., 2005; Rosypal et al., 2005). For the *Leishmania* IFAT, acetone-fixed *L. infantum* promastigotes (American Type Culture Collection, (ATCC #PRA-149), Manassas, VA) originally isolated from a naturally infected dog (Rosypal et al., 2003a) were employed as antigens. Brazil strain *T. cruzi* epimastigotes were used as antigen in the *Trypanosoma* IFAT. Positive and negative controls were used on each IFAT slide. Sera obtained from dogs with proven *T. cruzi* and *L. infantum* infections were used as positive controls for respective IFAT tests. Negative control serum used in both IFATs was obtained from a dog determined to be uninfected by both culture and serology. Sera reactive at a dilution of 1:25 were considered positive and positive samples were titrated. Slides were viewed with an Olympus BHT microscope equipped with BH2-RFC reflected light epifluorescent optics.

## 3. Results

A total of 365 dogs were tested in the current study for detectable IgG antibodies to *L. infantum*. Five of the 107 canine samples (4.7%) from Brazil were positive by IFAT. Two Brazilian dogs had IFAT titers of 1:25 and 2 were positive at 1:50. One Brazilian dog was seroreactive at a dilution of 1:100. Of the 258 dogs tested from Colombia, 4 (1.6%) had antibodies at titers of 1:25 to *L. infantum*.

Of the 365 serum samples assayed in this work, none of the Brazilian or Colombian dogs had IFAT titers to *T. cruzi*.

## 4. Discussion

We investigated the prevalence of antibodies to *L. infantum* and *T. cruzi* in 365 dogs from urban areas of Brazil and Colombia to provide epidemiological information on infection of these zoonotic parasites. We found 4.7% and 1.6% of dogs tested by IFAT from Brazil and Colombia, respectively, were positive for anti-*Leishmania* antibodies. None of the dogs had detectable antibodies to *T. cruzi*. Results obtained in the present study indicate a low or absent seroprevalence to *Leishmania* and *Trypanosoma* parasites, respectively, among dogs from these geographic regions.

A wide range of seroprevalence rates have been previously reported for anti-*Leishmania* antibodies in dogs in various parts of South America (Dantas-Torres et al., 2006b; França-Silva et al., 2003; Paranhos-Silva et al., 1996). In Brazil, antileishmanial antibody rates in urban

areas have ranged from 9.9% (França-Silva et al., 2003) to 40.3% (Dantas-Torres et al., 2006b).

Human VL was registered in 34 municipalities in the northeast region of the state of São Paulo, including a few cases in the cities of São João da Boa Vista and São Paulo (São Paulo, 2006). Although the city of São Paulo is not considered an endemic city, there have been increased reports of human VL cases in São Paulo State (Moreira et al., 2007 in press) which indicates VL expansion (França-Silva et al., 2003). Results from the current study, however, suggest that the prevalence of canine infections is low in these urban areas and may indicate limited efficacy of canine culling as a control measure for human VL in these geographic regions. Urbanization, migration, poor sanitary conditions, co-infection with HIV and malnutrition are risk factors which may be responsible for increased cases of zoonotic VL in Latin America (Desjeux, 2001).

Seroprevalence rates are dependent on the selected cut-off titer and technical considerations. Failure to seroconvert, false-positive and false-negative IFAT results can all occur in *Leishmania*-infected dogs (Rosypal et al., 2005) and determination of the true prevalence of infection may be influenced by these factors. Serum samples reactive at a dilution of 1:80 are considered positive by the Ministry of Health of Brazil (Brasil, 2004; Dantas-Torres et al., 2006b). For the present study, we used a cut-off titer of 1:25. Use of a higher cut-off point potentially underestimates the actual number of infected dogs, however it increases the specificity of the test and may prevent destruction of false-positive dogs (Dantas-Torres et al., 2006b).

Diagnosing canine VL is challenging due to variable clinical presentations and a high proportion of asymptomatic dogs. Diagnosis is achieved by parasitological, serological, or molecular methods. Demonstration of the parasite by microscopy or culture provides definitive proof of infection, but serology is the standard diagnostic tool for detecting *Leishmania*-infected dogs in endemic areas due to convenience, cost, and sensitivity considerations (Reithinger and Davies, 1999).

There are several serological methods used for detection of *Leishmania*-specific antibodies including the IFAT, complement fixation, direct agglutination, ELISA, and immunochromatographic dipstick tests (Rosypal et al., 2003b; Lira et al., 2006). The IFAT is the most commonly used of all the serological assays (Dantas-Torres, 2006b) and it is the “gold standard” by which other serological tests are measured (Rosypal et al., 2003b). The IFAT is the recommended test for use in canine surveys by the Brazilian Ministry of Health (Lira et al., 2006).

The overall sensitivity and specificity of IFAT employed in this work has been shown to be 89–92% and 27–29%, respectively, depending on cut-off values, in experimentally infected dogs (Rosypal et al., 2005). Cross-reactivity in the *T. cruzi* IFAT was previously reported among *Leishmania*-infected dogs with titers of as low as 1:25 (Rosypal et al., 2005), but cross-reactions were not observed in the current study.

A commercially available IFAT kit used in Brazil utilizes *L. major* antigens (Dantas-Torres et al., 2006b; Lira et al., 2006) and previous work indicates that antibody tests using antigens of different *Leishmania* species may impact serological results (Baleeiro et al., 2006; Dantas-Torres et al., 2006b). *Leishmania braziliensis*, a causative agent of American cutaneous leishmaniasis, is also endemic in Latin America. *L. braziliensis*-infected dogs may be seroreactive by IFAT leading to cross-reactivity in areas where both *Leishmania* species are present (Madierra et al., 2005). We cannot exclude the possibility that the dogs with detectable anti-*Leishmania* antibodies were infected with *L. braziliensis*. However, *L. infantum*

promastigotes were used as antigens in our IFAT and Baleeiro et al. (2006) suggests that test performance is improved by using antigens of the same species.

Several researchers have emphasized the utility of PCR for diagnosis of canine VL due to its high sensitivity and specificity (Moreira et al., 2007 in press; Gomes et al., 2007). Prevalence of infection is usually higher using molecular-based diagnostic methods than serological methods because many dogs are PCR-positive and serologically negative (Leontides et al., 2002). The sensitivity and specificity of the IFAT used in this work, however, is similar to PCR performed on dogs from Brazil which exhibited a sensitivity of 92.3% and specificity of 37.5% (Andrade et al., 2006). Canine culling programs to control VL in endemic areas, however, are based on serological tests and despite their use, there are increasing reports of human VL in parts of Brazil (Moreira et al., 2007 in press).

International initiatives to control Chagas' disease have significantly reduced the impact of disease burden in Latin America resulting in a 67% decrease incidence of new human infections with *T. cruzi* (Moncayo et al., 2006). Control interventions for American trypanosomiasis started in 1991 in contiguous endemic areas in an effort to eliminate insect vectors. By 2005, Brazil was certified free of *T. cruzi* transmission to humans (Moncayo et al., 2006) and in Colombia, 3 million known patients with Chagas' disease exist (Gutierrez et al., 2004). Indeed, even in areas where transmission has been interrupted, the normal life-cycle of *T. cruzi* involves domestic or sylvatic animals and triatomine bugs without a requirement for humans (Moncayo et al., 2006).

Dogs are a major source of *T. cruzi* infection in the domestic transmission cycle and although vector control measures reduce insect numbers, canine infections recover quickly (Gürtler et al., 2006). Estrada-Franco et al. (2006) demonstrated a correlation between seropositive dogs and humans. *T. cruzi* is divided into two main phylogenetic groups, *T. cruzi* I and II (Higo et al., 2004). *T. cruzi* I occurs in Colombia and *T. cruzi* I and II are present in Brazil (Higo et al., 2004). Both major genotypes are responsible for human and canine cases of American trypanosomiasis (Crisante et al., 2006).

Immunodiagnosis of *T. cruzi* is primarily based on detection of IgG antibodies by serological tests including indirect hemagglutination, ELISA, and IFAT (Moncayo et al., 2006). In one study comparing four serological assays, IFAT detected the highest number of seropositive individuals (Gutierrez et al., 2004). The use of multiple antigens, such as whole organisms used in the present IFAT, increases sensitivity over tests that use recombinant or single purified antigens (Moncayo et al., 2006). Additionally, it is recommended that serological tests should use autochthonous parasite strains for reliable serodiagnosis (Gutierrez et al., 2004). There is also a possibility of cross-reactions with *Leishmania* spp. and *Trypanosoma rangeli* in areas with overlapping geographic distribution (Gutierrez et al., 2004; Moncayo et al., 2006). In the present work, however, we did not observe any cross-reactivity in the *T. cruzi* IFAT.

Efforts to control *T. cruzi* have been successful in significantly impacting the incidence, morbidity and mortality associated with Chagas' disease (Dias et al., 2002). The biggest challenge facing the success of intervention programs is sustainability (Moncayo et al., 2006). Even in areas where transmission is interrupted, there is a risk of disease recrudescence (Dias et al., 2002). Animal reservoirs and humans immigrating into non-endemic areas with infected domestic animals could result in the spread of *T. cruzi* (Estrada-Franco et al., 2006). The need for active epidemiological surveillance programs is clear.

The low seroprevalence of antibodies to *L. infantum* and the lack of antibodies to *T. cruzi* found in the present study indicate that domestic dogs do not play an important role in the epidemiology of these zoonotic parasites in the urban areas studied in this work. Due to the public health concerns regarding these parasites, further epidemiological studies should be



conducted to determine the presence of *L. infantum* and *T. cruzi* in dog populations in other parts of Brazil and Colombia in order to determine the associated risk factors.

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