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Seroprevalence of Canine Leishmaniasis in Owned and Stray Dogs from Grenada, West Indies

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ABSTRACT

The aim of this study was to estimate the seroprevalence of Leishmania antibodies in two populations of dogs (owned and stray) in Grenada, West Indies. Leishmainiasis caused by a hemoflagelate protozoan, is zoonotic disease that affects a wide range of animals including man. Dogs are considered main reservoir for the organism. Antibodies to Leishmania spp were determined in serum samples from 836 dogs (344 stray and 492 owned dogs) using qualitative immunochromatographic dipstick tests (ICTs) based on recombinant antigens specific for visceral leishmaniasis (Kalzar detect rapid test:In Bios, USA). Seropositivity for leishamanis spp. was detected in 23 dogs (2.7%, 95% confidence Interval (CI) 1.06%to 3.8%). Thirteen of these were stray dogs (3.7%) and ten were owned dogs (2.0%). Results from this study indicate that dogs in Grenada are exposed at a low level to leishamaniasis.

Keywords: Antibodies, Grenada, West indies, Leishmania spp., owned and stray dogs.

Canine leishmaniasis was first identified in Europe in 1903 (Dereure *et al*., 1999). Recent studies indicate that it is expanding in continental climate areas and spreading to regions previously regarded as non-endemic (Ferroglio *et al.,* 2005). Leishmaniasis has been reported in the inter-tropical zones of America and Africa, and into the temperate regions of Latin America, Europe and Asia. The main causative agents are the protozoa of the genus *Leishmania* which are zoonotic and they affect numerous mammal species including humans, and transmitted by the bite of *Phlebotomine* sand flies (*Phlebotomus* spp. and *Lutozoumyia* spp.) (Grammicia, 2011). Human leishmaniasis is the third most important vector-borne disease after malaria and lymphatic filariasis (Solano-Gallego and Baneth, 2008). It is variably prevalent in South American countries, especially in Venezuela and Brazil where a high prevalence of canine infection is associated with high risk of human disease (Baneth *et al.,* 2008, Moreno and Alvar, 2002). Leishmaniasis has been classified by the World Health Organization (WHO) as one of the most neglected tropical diseases with an estimated yearly incidence of 1-1.5 million cases of Canine leishmaniasis and 500,000 cases of Visceral Leishmaniasis (Desjeux, 1996; Grammicia, 2011). In addition to dogs, wild canines, wolves, foxes, rodents, marsupials and primates are considered main wild hosts (Dantas-Torres *et al.,* 2012).

In North America cases of Leishmaniasis in dogs began to appear in 2000 (Monti, 2000) and in 2008, Leishmania positive fox hounds have been reported in 18 states of the USA and 2 provinces in Canada where no autochthonous human cases were reported and no sand fly transmission was demonstrated (Rosypal, 2005, Baneth *et al.,* 2008).

Leishmaniasis is endemic in most of Latin (South) America but little is known regarding this disease in the neighboring countries in the Caribbean. Among the Caribbean countries, Zeledon, (1992) reported autochthonous focus of cutaneous Leishmaniasis in Dominican Republic and the black rat was suspected as a wild reservoir. Sporadic cases of leishmaniasis in human population have been reported from Martinique, Trinidad and Guadeloupe. the other island countries in the Caribbean (Zeledon, 1992) Little is known of *Leishmania* spp. infection in Grenada, apart from a preliminary survey conducted on serum samples of 77 dogs during 2007 (Rosypal *et al.,* 2010). No evidence of Leishmania infection was detected in the survey.

This paper presents results of a serological survey for leishmania antibodies, conducted during 2010 through 2013 in two populations of dogs; owned and stray; in Grenada.

MATERIALS AND METHODS

The project was approved by Institutional animal care and use committee (IACUC).

Blood samples from 492 owned dogs were from those participating in free SGU vaccination clinics conducted as a part of One Health One Medicine program in all 6 parishes of the main land and two sister islands of Grenada. Signed dog owner consent was obtained for inclusion of their dogs in the study. Prior to sampling, all dogs were examined by a veterinarian and given a body condition score. Any dog exhibiting clinical signs of systemic disease, or if a blood sample could not be obtained, was excluded from the study. Samples from stray dogs were obtained from dogs captured from all six parishes of the country by the Ministry of Health in collaboration with the Ministry of Agriculture, Forestry and Fisheries, Government of Grenada under the "Stray dog control program". Clinical status and body condition for the stray dogs were not recorded. No stray dogs were received from the sister islands of Carriacou and Petit Martinique.

Blood from all dogs was collected from either the jugular, cephalic, or the lateral saphenous vein. Serum was separated by centrifugation at 1500g and stored frozen (-20°C) at the Pathology diagnostic laboratory, St. George's University until all samples were analyzed.

Commercial canine immunochromatographic (ICT) dipstick assays were conducted for qualitative antibody detection to *Leishmania spp*. Serum samples were tested for antibodies to recombinant K39 (rk39) (Kalazar Detect™ Canine Rapid Test, InBios International Ltd., Seattle, Washington) based on multiepitope recombinant antigens developed into a dipstick format. The test was performed according to the manufacturer's test procedure. Briefly, dog serum (20µL) was aliquotted onto a test strip. The dipstick then was placed in a well of 96-well round bottom tissue culture plate and provided chase buffer (100-150 µL). Test results were read after 10 minutes, a red control line and a second line in the test field appeared if the result was positive. The presence of a red band only on the control line indicated a negative result. Results were compared between owned and stray dogs with further analysis of age and sex related distribution using a chi-square statistical method.

RESULTS AND DISCUSSION

Seropositivity for *Leishmania* spp. was detected in a total of 23 dogs (2.7% 95% CI: 1.6% to 3.8%)), 13 of these were stray dogs (3.7%) and ten were owned dogs (2.0%). (Table 1).

Table 1: Seroprevalence of anti-Leishmania antibodies in stray and owned dogs from Grenada, West Indies.

	Pet dogs	**Stray dogs**
Total dogs tested	492	344
Total positive dogs	10	13
Percentage	2.0%	3.7%

There is no statistically significant difference (chi-square=.31), p-value=.86) between the proportion of pet and stray dogs that tested positive to Leishmania.

Majority of the dogs tested positive were in the age group of 2 years to 4 years (3.6 %) and < 2 years (3.3 %). Only one dog in the older age group (> 5 years) tested positive (1.1%), (Table 2).

Table 2: Age wise seroprevalence of anti-Leishmania antibodies in stray and owned dogs from Grenada, West Indies

Age groups	Pet dogs			Stray dogs		
	Total tested	Positive	Percentage	Total tested	Positive	Percentage
< 2yrs	158	05	3.1 %	23	01	4.3 %
2yrs – 4 yrs	224	04	1.7 %	221	12	5.4 %
> 5 yrs	100	01	1.0 %	0	0	0

There was no statistical difference between age and testing positive (chi=0.77, p=0.68).

Of the total dogs tested 399 were male dogs and 473 were female dogs with seroprevalence of 2.5 % and 2.9 % respectively (Table 3)

Table 3: Sex wise seroprevalence of anti-Leishmania antibodies in stray and owned dogs from Grenada, West Indies

Sex	Pet dogs			Stray dogs		
	Total tested	Positive	Percentage	Total tested	Positive	Percentage
Male dogs	257	07	2.7 %	142	03	2.1 %
Female dogs	235	03	1.2 %	202	10	4.9 %

There is no statistically significant gender difference (chi-square=.68, p-value=.71) between the proportion of dogs testing positive for leishmania antibodies..

Overall seropositivity of Leishmania spp in Grenada was 3.7% in Stray dogs and (2.0%) in owned dogs. There was no significant difference in seropositivity of two populations of dogs ($p>0.05.x^{2)}$. A few researchers have found higher proportion of stray dogs with antibodies to Leishamania spp. compared to owned dogs. Cortes *et al.*,(2007), reported 18.4% infection in domestic dogs compared to 21.65% in stray dogs in Portugal during 2002-2003. A similar trend was reported by Miro *et al.,* (2007) in the region of Madrid and by Gavgani *et al.,* (2002) from northwest areas of Iran. A significantly higher (>0.05 x^2) seroprevalence was recorded by Hosseininejad *et al.,* (2012) in stray dogs from 3 Iranian provinces as compared to owned dogs (9.4%). Contrary to this statement, others have reported absence or low infection of leishmaniasis in stray dogs. Khanmahammadi *et al.,* (2009) found all 150 stray dogs negative for leishsmania antibodies in Marand city of Iran, while during 2008-9, they reported 3.3% to 29.6% infection in owned dogs in various areas of Iran. Haddadzade *et al.,* (2013) found 10% seroprevalence among owned dogs as compared to 1.6% in stray dogs from Alborz province of Iran In a similar study by Savani *et al.,* (1999) in Brazil, all 973 stray dogs were negative for leishmania antibodies.

The equal level of exposure to Leishmania spp in owned and stray dogs found in Grenada could be because of the husbandry and pet keeping practices by dog owners in this country, contrary to New World countries. In Grenada majority of dogs are kept as watch dogs. They are fed and looked after well but not allowed to sleep and shelter inside the house. These dogs are prone to flea bite similar to stray dogs.

There are at least 12 species of *Leishmania*, but the most important etiological agent of Canine Leishmaniasis (CanL) is *Leishmania infantum (Syn. L.chagasi)* (Dantas-Torres *et al*., 2012). Dogs being the main reservoir of zoonotic visceral leishmaniasis, it causes fatal infections in humans and dogs.

Dogs may suffer from a complex syndrome (CanL) which can be a challenge to diagnose clinically as it has a prolonged incubation period (months to years) with variable clinical signs which include dermatpathy, lymphadenopathy, epistaxis, weight loss, ocular and renal abnormalities. Furthermore, many dogs may remain asymptomatic without having the clinical disease (Marcondes *et al.,* 2011; Baneth and Aroch, 2008). Canine infections are epidemiologically considered more important because both seropositive asymptomatic and symptomatic dogs are similarly infectious to Phlebotomine vectors (Grammicia *et al.,* 2010).

Any owned dog, with clinical signs was excluded from the study hence seropositive dogs in this group may be asymptomatic. However, exclusion was not possible in stray dogs where clinical examination was not performed.

In our study the majority of the seropositive dogs were from the young (3.3%) or young adult age groups (3.6%). Although not significant statistically, but on comparison between the stray and owned dogs higher seroprevalence was noticed among the 2yrs to 4 yrs old stray dogs than those of owned dogs (1.7%). Similar observations were made by Galvez *et al.*,(2010) and Sideris *et al.,* (1999). But in our study only one dog from the older age groups (1.1%) showed seropositive results which was in contrast to their observations. Overall, majority of the seropositive dogs were female (13/437), but no significant difference noticed from the numbers of seropositive male dogs (10/399).

Many species of *Phlebotomus* sand flies have been incriminated as vector of leishmaniasis in different countries of the world. However, *P. ariasi* and *P. pernicious* are known to be more involved in disease transmission being preferentially zoophilic. Large number of *Phlebotomus* sand flies (mostly *Lutzomyia spp.)* infests Caribbean Islands (Zeledon, 1992; Tilaksingh, 2011; Young and Duncan 1994), but no reports of presence of any particular Phlebotomine sand flies are available for Grenada. As per our knowledge no major entomological studies were conducted in the last 20 years to determine the presence of these vectors in Grenada. With flourishing tourism and education centers, Grenada has experienced an increased human migration, sometimes accompanied by dogs from different parts of the world in last 10 years. Possible spread of *Leishmania spp.* from countries with known leishmania and sand flies prevalence to non-endemic countries in this region is expressed by Zeledon, (1992), It may be of concern for Grenada which is in a close proximity of such countries and possess similar climate and landscape features.

Although sand flies are the only biological vectors of leishmaniosis, more recently other ectoparasites such as ticks and fleas have been proposed as possible vectors (Dantas-Torres, 2011; Ferreira *et al.*,2009; Paz *et al.,* 2010), but their significance has not been verified in natural condition (Baneth *et al.,* 2008, Otranto and Dantes-Torres, 2010). Duprey *et al.,* (2006) suggested a possible dog-to-dog transmission while explaining leishmania positive canine cases from different US states where presence of sand-fly vectors was not proven.

The ICT dipsticks used in this study are based on recombinant antigens and are considered ideal diagnostic tools for testing in remote areas (Chappuis *et al.,* 2007). The ICT is a qualitative assay, but it detects canine antibodies to *Leishmania spp.* with titers as low as 1:50 (Rosypal, 2005). In addition, several studies using the recombinant antigens in the ICT demonstrated high specificity compared to traditional serological assays (Bern *et al.,* 2000; Otranto *et al.,* 2004; Burns *et al.,*1993; Rosypal, 2005). Whereas a sensitivity of 70-77%, comparable with ELISA techniques, has been reported by Reithinger *et al.*,(2002) and Mettler *et al.,* (2005). Serological cross reactivity with *Trypanosoma cruzi* and other infectious diseases could be a possibility, but the recombinant antigen used in ICT tests minimizes those chances (Mettler *et al.,* 2005). Furthermore, none of the leishmania seropositive dogs were positive for other diseases like *Trypanosoma spp, Toxoplasma gondii* and *Neospora caninum* which were tested as a part of a different study on the same samples (Chikweto *et al.,* 2014). Although 3 of the positive dogs were also positive for heartworm and two dogs had Transmissible Venereal Tumor lesions, but we are unaware of their effect on ICT tests.

Following the report of negative seroprevalence on 77 dogs by Rosypal *et al.,* (2010) in Grenada, the present study serves as the first known documentation of positive canine leishmaniasis from the island of Grenada. This will help to increase awareness for this zoonotic disease in veterinarians and dog owners, as well as institution of regular disease surveillance by local public health authorities.

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REFERENCES

Baneth, G. and Aroch, I. 2008. Canine leishmaniasis: A diagnostic and clinical challenge. *The Vet. J.,* **175:** 14–15.

Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P. and Ferrer, L. 2008. Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part one. *Trend Parasitol.,* **24**(7): 324-330

Bern, C., Jha, S.N., Joshi, A.B., Thakur, G.D. and Bista, M.B. 2000. Use of the recombinant K39 dipstick test and the direct agglutination test in a setting endemic for visceral lesihmaniasis in Nepal. *Am. J. Trop.Med. Hyg.,* **63 (3 and 4):** 153-157.

Burns, J.M., Shreffler, W.G., Benson, D.R., Ghalib, H.W., Badaro, R. and Reed, S.G., 1993. Molecular characterization of a kinensin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral lesihmaniasis. *Proc. Natl. Acad. Sci. USA,* **90:** 775-779.

Carribean Development Report. 2011. The economics of Climate change in the Caribbean. United Nations Economic Commission for Latin America and the Caribbean.**3**.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, RW, Alvar, J., Boelaert, M., 2007. Visceral lesihmaniasis: what are the needs for diagnosis, treatment and control. *Nature Rev. Microbiol.* **5:** 873-882.

Chikweto, A., Kumthekar, S., Chawla, P., Tiwari, K.P., Perea, L.M., Paterson, T., Sharma, R.N., 2014. Seroprevalence of *Trypanosoma cruzi* in stray and pet dogs in Grenada, West Indies. *Tropical Biomedicine,* **31(2):** 1-4.

Cortes, S., Alfonso, MO, Alves-Pires, C., Campino, L., 2007. Stray dogs and Leishmaniasis in urban areas, Potugal. *Emerg. Inf. Dis.* **13 (9):** 1431-1432.

Dantas-Torres, F., 2011. Ticks as vectors of *Leishmania* parasites.*Trend Parasitol*. **27(4):** 155-159.

Dantas-Torres, F, Brito, MEF, Brandao-Filho, SP, 2006. Seroepidemiological survey on canine Leishmanisis among dogs from an urban area of Brazil. *Vet Parasitol.* **140:** 54-60.

Dantas-Torres, F., Solano-Gallego, L., Baneth, G., Ribeiro, VM., Paiva-Cavalacanti, M., Otranto, D., 2012. Canine leishmaniosis in the old and New Worlds: Unveiled similarities and differences. *Trend Parasitol,* **28 (**12): 531-538.

Dereure, J, Pratlong, F, Dedet, JP. 1999, Geographical distribution and the identification of parasites causing canine Leishmaniasis in the Mediteranian Basin. Canine Leishmania-an update. Proceedings of the International Canine Leishmaniasis, Forum, Barcelona, Spain.

Desjeux, P., 1996. Lesihmanisis. Public health aspects and control. *Clin. Dermatol.* **14,** 417-423.

Duprey Z.H., Steurer F.J., Rooney J.A., Kirchhoff L.V., Jackson J.E., Rowton E.D., 2006. Canine visceral leishmaniasis, United states and Canada, 2000-2003. *Emerg Inf Dis,* **12(3):** 440-446.

Ferreira, MGPA, Fattori, KR, Souza, F., Lima, VMF, 2009. Potential role for dog fleas in the cycle of *Leishmania* spp. *Vet Parasitol* **165:**(1–2): 150–154

Ferroglio, E, Maroli, M, Gastaldo, S, Mignone, W, Rossi, L. 2005. Canine Leishmaniasis Italy. *Emerg Infect Dis* **12**(10): 1618-20.

Gavgani, ASM, Mohite, H., Edrissian, GH, Mohebali, M., Davies, CR, 2002.Domestic dogownership in Iran is a risk factor for human infection with *Lesihmania infantum*. *Am. J. Trop. Med. Hyg.,* **67**(5): 511-515.

Grammicia, M., 2011. Recent advances in lesihmaniosis in pet animals: Epidemiology, diagnostics and anti-vetorial prophylaxis. *Vet Parasitol* **181:** 23-30.

Gramiccia,M., Muccio,T., Fiorentino, E., Scalone, A., Bongiorno, G., Cappiello, S., Paparcone, R., Manzillo, VF, Maroli, M., Gradoni, L., Oliva, G., 2010. Longitudinal study on the detection of canine Leishmania infections by conjunctival swab analysis and correlation with entomological parameters. *Vet Parasitol* **171:** 223–228.

Gálvez,R., Miró, G., Descalzo, MA, Nieto, J., Dado, D., Martín, O., Cubero, E., Molin, R., 2010. Emerging trends in the seroprevalence of canine leishmaniosis in the Madrid region (central Spain). *Vet Parasitol* **169** (3–4): 327–334.

Haddadzade, HR, Fattahi, R., Mohebali, M., Akhoundi, B., Ebrahimzade, E., 2013. Seroepedimiological Investigation of Visceral Lesihmaniasis in Stray and Owned Dogs in Alborz province, Central Iran using Direct Agglutination Test. *Iranian J. Parasitol,* **8**(1)**:** 152-157.

Hosseininejad, M., Mohebali, M, Hosseini, F., Karimi, S., Sharifzad, S., Akhoundi, B., 2012. Seroprevalence of canine visceral lesihmaniasis in asymptomatic dogs in Iran. *ijvr SHIRAZU* **13**(1): 54-57.

Khanmohammadi, M, Sadaghian, M, Babaey Neghad, F, Zakaria, M., 2009. Study on seroprevalence of visceral Leishmaniasis in Stray dogs of marand (East Azerbiajan) with indirect immunoflorescence test (IFAT) and its health importance in 2007-2008. *The Internet J. Parasitic disea,* **4**(1).

Marcondes, M., Biondob, AW, Gomes,AAD, Silva, ARS, Vieirae, RFC, Camacho, AA, Quinn, J., Chandrashekar, R., 2011. Validation of a Leishmania infantum ELISA rapid test for serological diagnosis of *Leishmania chagasi* in dogs. *Vet. Parasit.* **175:** 15–19.

Mettler, M., Grimm, F., Capelli, G., Camp, H., Deplazes, P., 2005. Evaluation of enzyme-linked immunosorbent assays, an immunofluorescence-antibody test, and two rapid tests (immunochromatographic-dipstick and gel tests) for serological diagnosis of symptomatic and asymptomatic Leishmania infection in dogs. *J. Clin. Microbiol.* **43:** 5515–5519

Miro, G, Montoya, A, Mateo, M, Alonso, A, Garcia, S, Garcia, A., 2007. A Leishmaniasis surveillance system among stray dogs in the region of Madrid: ten years of serodiagnosis (1996-2006). *Parasitol Res*. **101:** 253-257.

Monti, Dean (2000) Hunters hounded as Leishmaniasis is diagnosed in foxhounds. *J. Am. Vet. Med. Assoc.* **216 (12):** *1887-1890.*

Moreno, J., Alvar, J.,2002. Canine leishmaniasis: epidemiological risk and the experimental model. *Trend Parasitol* **18 (9):** 399-405

Otranto, D., Dantes-Torres, F., 2010. Fleas and ticks as vectors of *Leishmania* spp. To dogs: caution is needed (Letter to the Editor). *Vet Parasitol* **168:** 173-174.

Otranto, D., Paradies, P., Sasanelli, M., Spinelli, R., Brandonisio, O., 2004. Rapid immunochromatographic test for serodiagnosis of canine lesihmaniasis. *J. Clin Microbiol,* **42 (6):** *2769-2770.*

Paz, GF, Ribeiro, MFB, de Magalhães,DF, Sathler, KPB, Morais, MHF, Fiúza, VOP, Brandão, ST, Werneck, GL, Fortes-Dias CL, Dias, ES.,2010. Association between the prevalence of infestation by *Rhipicephalus sanguineus* and *Ctenocephalides felis felis* and the presence of anti-*Leishmania* antibodies: A case–control study in dogs from a Brazilian endemic area. *Prev Vet Med,* **97(2):** 131–133.

Reithinger, R., Quinnell, RJ, Alexander, B., Davies, CR, 2002. Rapid Detection of *Leishmania infantum* Infection in Dogs: Comparative Study Using an Immunochromatographic Dipstick Test, Enzyme-Linked Immunosorbent Assay, and PCR, *Journal. Clin. Microbiol.* **40 (7):** 2352–2356.

Rosypal, A, 2005. Characterization of canine Leishmaniasis in the United States: Pathogenesis, immunological response and transmission of an American Isolate of L. infantum. Dissertation submitted to the Faculty of the Virginia Polytechnic Institute & State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy In Veterinary Medical Sciences, Blacksburg, VA.

Rosypal, A., Tripp, S., Kinlaw, C., Sharma, RN, Stone, D., Dubey, JP, 2010. Seroprevalence of Canine Leishmaniasis and American Trypanosomiasis in dogs from Grenada, West Indies. *J. Parasitol.,* **96 (1):** *228-229.*

Savani, ES, Galati, EA, Camargo, MC, D'Auria, SR, Domaceno, JT, Baldvino, SA (1999) Serological survey for American cutaneous Leishmaniasis in stray dogs in the S. Paulo state, Brazil. *Rev. Soude Publica* **33(6):** 629-31.

Sideris V.,Papadopoulu G., Dotsika E. and Karagouni E., 1999. Asymptomatic canine leishmaniasis in Greater Athens area, Greece. *Euro J Epidemiol*, **15:** 271-276.

Solano-Gallego L. and Baneth, G., 2008. Canine leishmaniosis – a challenging zoonosis. EJCAP*,* **18 (3):** 232-241.

Tilaksingh, ES, 2011. Collection of Phlebotomine sand flies (Diptera: Psychodidae), 1953-1977 in Trinidad, West Indies. Living World, *J.Trinidad and Tobago Field Naturalists Club.*

Young, D.G. and Duncan, M.A. 1994. Guide to the Identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Memoirs of the American Entomological Institute, No.54. Associated Publishers, Florida, USA.

Zeldon, R., 1992. Leishmanisis in the Caribbean islands: A review, *Ann NY Acad Sci,* **16**: 154-60.