**Revista da Sociedade Brasileira de Medicina Tropical**
*Journal of the Brazilian Society of Tropical Medicine*
**Vol.:52:e20180133: 2019**
doi: 10.1590/0037-8682-0133-2018

## Short Communication

# Circulation of spotted fever group rickettsiae among dogs seropositive for *Leishmania* spp. in an urban area of Brazil

***Sabrina Destri Emmerick Campos*[1], *Niara Vanat Nadal*[1], *Helena Keiko Toma*[2], *Adilson Benedito de Almeida*[3], *Matheus Dias Cordeiro*[4], *Adivaldo Henrique da Fonseca*[4], *Fabiano Borges Figueiredo*[3], *Maurício Afonso Verícimo*[5], *Nathalie Costa da Cunha*[6] and *Nádia Regina Pereira Almosny*[1]**

[1]. Departamento de Patologia e Clínica Veterinária, Universidade Federal Fluminense, Niterói, RJ, Brasil.
[2]. Laboratório de Diagnóstico Molecular e Hematologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.
[3]. Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil.
[4]. Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil.
[5]. Departamento de Imunologia, Universidade Federal Fluminense, Niterói, RJ, Brasil.
[6]. Departamento de Saúde Coletiva Veterinária e Saúde Pública, Universidade Federal Fluminense, Niterói, RJ, Brasil.

**Abstract**

**Introduction:** Dogs play an epidemiological role in several vector-borne diseases that affect human and animal health worldwide. We aimed to identify rickettsial circulation among dogs with canine visceral leishmaniasis (CVL) from a region endemic for both diseases. **Methods:** CVL-seropositive dogs were screened for spotted fever group rickettsiae using an indirect immunofluorescence assay. **Results:** Among the CVL-positive dogs, anti-*Rickettsia rickettsii* antibodies were identified in one asymptomatic and one oligosymptomatic dog. **Conclusions:** This study shows low circulation of antibodies to *R. rickettsii* in CVL-seropositive dogs. It is recommended that surveillance studies in dogs should continue in order to monitor this scenario.

**Keywords:** Indirect immunofluorescence assay. Vector-borne diseases. Zoonosis.

It is estimated that vector-borne diseases account for approximately 17% of infectious diseases worldwide, being especially prevalent in impoverished tropical regions with poor sanitation[1]. One such disease is visceral leishmaniasis, caused by the parasite *Leishmania infantum* (syn. *L. chagasi*). In urban environments, domestic dogs are the main reservoir of this zoonosis[2]. The parasite is transmitted by phlebotomine insects belonging to the genus *Lutzomyia* (New World) and *Phlebotomus* (Old World)[2]. Phlebotomines related to visceral leishmaniasis are mainly *Lutzomyia longipalpis* and *L. cruzi*[2]. *Lutzomyia longipalpis* has a wide geographical distribution and seems to be well adapted to the urban environments of the southeast region of Brazil, living in and around human domiciles, henhouses, pigsties, and kennels[2].

Another vector-borne disease with an impact on public health is Brazilian spotted fever (BSF), a highly fatal disease caused by the bacterium *Rickettsia rickettsii*[3]. The main vectors of *R. rickettsii* in Brazil are ticks of the *Amblyomma cajennense* species complex (*A. cajennense* sensu lato), followed by those of the species *A. aureolatum*. *Rhipicephalus sanguineus* is a suspected vector and may play a role in transmission in certain situations[3]. Dogs, which live in close proximity to humans and many possible vectors, are considered reservoirs for canine visceral leishmaniasis (CVL) and good sentinels of BSF[4].

Barra Mansa (22°32′39″ S, 44°10′17″ W) is a Brazilian municipality in the south of the state of Rio de Janeiro, with a mild climate (annual mean temperature 20.7 °C), rainy summers, and Atlantic Forest vegetation. Human cases of visceral leishmaniasis, with high mortality rates, have been reported in

***Corresponding author:*** Sabrina Destri Emmerick Campos.
**e-mail:** s.destri@gmail.com
**Orcid:** 0000-0002-2703-3211
**Received** 24 April 2018
**Accepted** 24 July 2018

this municipality[5]. In addition, Barra Mansa is within the Vale do Paraíba region, an area where human cases of BSF have been reported since 2007[6], making the region endemic for both diseases. This has raised awareness of the need for surveillance in dogs, which should be screened for CVL and BSF.

Given the zoonotic potential of CVL and BSF together with the participation of dogs in their epidemiological cycles, the aim of this study was to verify the presence of anti-*Rickettsia* spp. antibodies in dogs with a previous diagnosis of CVL that lived in a region endemic for both diseases, independent of clinical symptoms. The development of this study was approved by the Ethics Committee on Animal Research of the Universidade Federal Fluminense (CEUA 438/2013 and 525/2014).

This study included 26 dogs, unvaccinated against CVL, that tested positive for *Leishmania* spp. on a dual-path platform immunochromatographic test (DPP rapid test; Bio Manguinhos/Fundação Oswaldo Cruz, Rio de Janeiro, Brazil) and on an enzyme-linked immunosorbent assay for CVL (EIE-LVC; Bio Manguinhos/Fundação Oswaldo Cruz). These dogs were sent to the Fundação Oswaldo Cruz by the municipal government of Barra Mansa for euthanasia; prior to the euthanasia, the animals were submitted to physical examination and collection of blood samples.

Classification according to the presence of typical CVL clinical signs was performed as previously suggested[2]. The dogs were classified as asymptomatic (absence of clinical signs); oligosymptomatic (discrete clinical manifestations and adenopathy); or symptomatic (presenting all or some of the more common signs of the disease).

Blood samples were centrifuged and sera were diluted to 1:64 in phosphate buffered saline to perform an indirect immunofluorescence assay (IFA), which was conducted as previously described[7]. Slides used for IFA were prepared with *R. rickettsii* str. Taiaçu, and *R. parkeri* str. AT-24 infected vero cells[8,9]. Serum samples showing uniform and coccoid-, bacillary-, or coccobacillary-shaped fluorescent spots at 1:64 titer were considered to be positive[7]. The frequency of dogs with a positive serological reaction was calculated; variables were stored in an electronic worksheet (EXCEL 2007, Microsoft®, São Paulo, São Paulo, Brazil) and analyzed using Fisher's exact test (BioEstat, version 5.0, Instituto Mamirauá, Belém, Pará, Brazil).

For data analysis, the animals were grouped as dogs with absence of CVL clinical signs (asymptomatic dogs), and dogs presenting clinical manifestations (symptomatic and oligosymptomatic). Asymptomatic dogs accounted for 19.2% of the present study, while symptomatic and oligosymptomatic dogs together accounted for 80.8%. Among these dogs, the most common clinical abnormality was onychogryphosis (53.8%), followed by poor physical condition and exfoliative dermatitis (50.0%); dehydration and skin lesions (42.3%); hypertrophy of the lymph nodes (38.5%); pallor of mucous membranes (30.8%); and hepatosplenomegaly (23.1%).

Of the 26 CVL-positive dogs, two (7.7%) presented a serological reaction to *R. rickettsii*, at a titer of 1:64. Although no immunoglobulin G (IgG) antibodies against *R. parkeri* were detected, it has been already demonstrated that there is an association between the seroreactivity for these two antigens by IFA and it is therefore not possible to identify the species involved[4].

No ticks were found on the animals at the time of sampling, although tick ectoparasitism had been consistently reported by the dog owners. Of the two animals in which anti-*R. rickettsii* IgG antibodies were detected, one was asymptomatic at the time of sampling, without obvious signs of rickettsiosis or CVL, and the other was oligosymptomatic, presenting nonspecific clinical signs (pallor of mucous membranes and poor physical condition). There was no significant difference ($p = 1.000$) between dogs seroreactive for *R. rickettsii* and the presence of clinical signs for CVL (asymptomatic and symptomatic/oligosymptomatic).

The most common symptoms in the present study were also reported by other authors[10,11], which emphasizes that skin lesions, onychogryphosis, and lymphadenopathy, for example, are promptly recognized in cases of CVL and are typical signs of disease in endemic regions. The presence of hepatosplenomegaly in 23.1% of the dogs is in agreement with studies that showed spleen injury in dogs with CVL[11]. Ultimately, we cannot rule out the fact that the clinical signs observed in the present study may also be related to other diseases of dogs, which unfortunately were not investigated.

Given that CVL is a severe, debilitating disease for dogs, we expected that dogs testing positive for *Leishmania* spp. would also present a serological response to other diseases. However, in our study, the proportion of dogs with anti-spotted fever group (SFG) rickettsiae antibodies was low. Similarly, Souza and Almeida[12] found a rate of co-infection between CVL and canine monocytic ehrlichiosis of only 2.5%.

Even though this was a limited study, we suggest that living in an area endemic for BSF is the most important determinant of seropositivity for SFG rickettsiae. One recent study showed that *R. rickettsii* antibody titers tend to be very high among dogs living in an area where there has been a recent outbreak of human BSF[13], which was not the case in the present study.

To our knowledge, the present study is the first to detect a serological reaction to *R. rickettsii* and *Leishmania* spp. simultaneously among dogs in Barra Mansa. Interestingly, in addition to the expansion and urbanization of *Leishmania* spp.[2], epidemiological data also suggest urbanization of SFG-*Rickettsia* spp.; this has been reported previously in a study that did not observe any significant difference between the frequency of anti-*Rickettsia* spp. antibodies in regions with different degrees of environmental preservation[4]. It is noteworthy that for both CVL and rickettsiosis, asymptomatic dogs may be important links in the epidemiological chain, as they are highly competent in establishing the infection of the vectors[14].

CVL and BSF are both vector-borne diseases; although these vectors are distinct on a first analysis, it has been proposed that *R. sanguineus* might be involved in the epidemiology of CVL[15], which is supported by the persistence of *L. infantum* kinetoplast DNA in unfed nymphs and adults of *R. sanguineus* and in the association between infestation by this tick and the

anti-*Leishmania* antibodies in dogs. In the present study, we suggest that CVL and BSF are probably diseases with peculiar characteristics. It is likely that there is no epidemiological overlap between the two, and the diagnosis of one has no influence on the risk of the other.

We believe that the municipality of Barra Mansa has low circulation of antibodies to *R. rickettsii*. Even so, we reinforce the concept that dogs serve as sentinels for the presence of SFG rickettsiae and we recommend that serological studies in dogs should continue in order to monitor this scenario.

**Acknowledgments:** Authors wish to extend gratitude to the *Superintendência de Controle de Endemias* of the state of São Paulo (SUCEN) and to the *Laboratório de Doenças Parasitárias*, UFRRJ for the supply of *Rickettsia* strains; and to *Laboratório de Pesquisa Clínica em Dermatozoonoses*, FIOCRUZ, for support in the diagnosis of leishmaniasis. Authors are grateful to Precise Editing for English-language editing of this manuscript.

**Financial support:** This study received financial support from the Brazilian *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, National Council for Scientific and Technological Development) and the *Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ, Foundation for the Support of Research in the State of Rio de Janeiro).

**Conflicts of interest:** There are no conflicts of interest to declare.

## REFERENCES

1. WHO. World Health Organization, Regional Office for South-East Asia. Vector-borne diseases. Report of an informal expert consultation SEARO, New Delhi, 7–8 April 2014. New Delhi: WHO Regional Office for South-East Asia; 2014. 39 p.
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de Vigilância e Controle da Leishmaniose Visceral. 1st ed. Brasília: Editora do Ministério da Saúde, Brasília – DF; 2006. 120 p.
3. Szabó MPJ, Pinter A, Labruna MB. Ecology, biology and distribution of spotted fever tick vectors in Brazil. Front Cell Infect Microbiol. 2013; 3: 1-9.
4. Campos SDE, Cunha NC, Machado CSC, Souza TVT, Fonseca ABM, Pinter A, et al. Circulação de Rickettsias do Grupo da Febre Maculosa em cães no entorno de Unidades de Conservação Federais do estado do Rio de Janeiro: evidência sorológica e fatores associados. Pesq Vet Bras. 2017;37(11):1307-12.
5. Pimentel MIF, Alves ELM, Silva MHFF, Moza PG, Mello CX, Schubach AO, et al. High lethality of visceral leishmaniasis in Barra Mansa, a new area of transmission in the state of Rio de Janeiro, Brazil. Rev Soc Bras Med Trop. 2014; 47(4):521-3.
6. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Sistema de Informação de Agravos de Notificação, Sinanweb [online]. 2015. [cited 2018 feb 11]. Available from: http://www.portalsinan.saude.gov.br/febre-maculosa
7. Horta MC, Labruna MB, Sangioni LA, Vianna MCB, Gennari SM, Galvão MAM, et al. Prevalence of antibodies to spotted fever group rickettsiae in humans and domestic animals in a Brazilian spotted fever-endemic area in the state of São Paulo, Brazil; Serologic evidence for infection by *Rickettsia rickettsii* and another spotted fever group rickettsia. Am J Trop Med Hyg. 2004;71(1):93-7.
8. Pinter A; Labruna MB. Isolation of *Rickettsia rickettsii* and *Rickettsia bellii* in cell culture from the tick *Amblyomma aureolatum* in Brazil. Ann N Y Acad Sci. 2006; 1078:523-9.
9. Silveira I, Pacheco RC, Szabó MP, Ramos HG, Labruna MB. *Rickettsia parkeri* in Brazil. Emerg Infect Dis. 2007;13(7):1111-3.
10. Marzochi MCA, Sabroza PC, Toledo LM, Marzochi KBF, Tramontano NC, Rangel Filho FB. Leishmaniose visceral na cidade do Rio de Janeiro - Brasil. Cad Saúde Pública. 1985;1(1):5-17.
11. Ciaramella P, Oliva G, Luna RD, Gradoni L, Ambrosio R, Cortese L, et al. A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. Vet Rec. 1997;141(21):539-43.
12. Souza VRF, Almeida ABPF. Co-infecção entre leishmaniose visceral e ehrlichiose monocítica em cães de Cuiabá, Mato Grosso. Acta Sci Vet. 2008; 36(2):113-7.
13. Poubel IT, Cunha NC, Fonseca ABM, Pinter A, Fonseca AH, Cordeiro MD, et al. Seroprevalence of *Rickettsia rickettsii* and *Rickettsia parkeri* in dogs during a Brazilian Spotted Fever outbreak in the State of Rio de Janeiro. Arq Bras Med Vet Zootec. 2018;70(3):667-74.
14. Laurentti MD, Rossi CN, Matta VLR, Tomokane TY, Corbett CEP, Secundino NFC, et al. Asymptomatic dogs are highly competent to transmit *Leishmania (Leishmania) infantum chagasi* to the natural vector. Vet Parasitol. 2013;196(3-4):296-300.
15. Paz GF, Ribeiro MFB, Magalhães DF, Sathler KPB, Morais MH, Fiúza VOP, et al. 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. 2010;97:131-3.