

Rickettsia in Synanthropic and Domestic Animals and Their Hosts from Two Areas of Low Endemicity for Brazilian Spotted Fever in the Eastern Region of Minas Gerais, Brazil

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Abstract. The aim of this study was to understand the current epidemiology of rickettsial diseases in two rickettsial-endemic regions in Brazil. In the municipalities of Pingo D'Água and Santa Cruz do Escalvado, among serum samples obtained from horses and dogs, reactivity by immunofluorescent assay against spotted fever group rickettsiae was verified. In some serum samples from opossums (*Didelphis aurita*) captured in Santa Cruz do Escalvado, serologic response against rickettsiae was also verified. Polymerase chain reaction identified rickettsiae only in ticks and fleas obtained in Santa Cruz do Escalvado. Rickettsiae in samples had 100% sequence homology with *Rickettsia felis*. These results highlight the importance of marsupials in maintenance of the sylvatic cycle of rickettsial disease and potential integration with the domestic cycle. Our data also support the importance of horses and dogs as sentinels in monitoring circulation of rickettsiae in an urban area.

INTRODUCTION

The rickettsial diseases are a group of diseases of worldwide importance that are caused by obligatory intracellular, Gram-negative bacteria of the genus *Rickettsia*. Most rickettsial species that are pathogenic to humans, and non-pathogenic rickettsiae, have arthropods (fleas, ticks, and lice) as vectors and reservoirs. In Brazil, the most prevalent rickettsiosis is Brazilian spotted fever (BSF), which is caused by *Rickettsia rickettsii*. Studies have shown that BSF is transmitted to humans by *Amblyomma cajennense* and *A. aureolatum* ticks.^{1,2} Several species of wild animals, including tapirs, rodents, birds, capybaras, snakes, bats, fish, and opossums, have been incriminated as hosts of rickettsial diseases. Pets such as dogs, cats, horses, and birds have also been considered as potential sources of infection.^{1,3,4}

Two counties in Minas Gerais, Brazil have had a history of rickettsial diseases. The municipality of Pingo D'Água, located in Vale do Rio Doce, is characterized by recent cases of BSF. The municipality of Santa Cruz do Escalvado, located in Vale do Piranga, Zona da Mata Mineira, has a history of rickettsial diseases but has undergone recent changes in its natural landscape because of construction of a hydroelectric plant in Soberbo in 2004. This region is considered susceptible for an outbreak of BSF.

To better understand the current epidemiology of rickettsial diseases in these municipalities, with an emphasis on occupation and environmental transformation caused by human activities in these regions, we assessed transmission of rickettsial agents in populations of domestic and synanthropic animals by serologic and molecular tests.

MATERIALS AND METHODS

We captured domestic (horses and dogs) and wild (opossums) animals to obtain serum samples and ectoparasites in

Pingo D'Água during July 2005–May 2006, and in Santa Cruz do Escalvado during June 2006–December 2007. Both sites were monitored every three months. In addition to ectoparasites, whole blood and anal swab specimens were obtained from opossums in Santa Cruz do Escalvado. This site is the only one in which opossums were captured.

DNA from blood samples, ectoparasites, and anal swab specimens was extracted as described.^{5,6} DNA was amplified by polymerase chain reaction using primers specific for the rickettsial citrate synthase gene (*gltA*), CS-62 (5'-GCA AGT ATC GAG GGT GAT GTA AT-3'), and CS-462 (5'-CTT CCT TCA TAA AAT ATA AAT CAG GAT G-3'), which amplify a 401-base pair fragment as described.⁷ Amplified products were visualized after electrophoresis on 1.5% agarose gels to confirm the reaction. The PCR products were purified by using the ExoSAP-IT Kit (United States Biochemicals, Cleveland, OH) and subjected to sequencing reactions using the Terminator Big Dye Kit (Perkin Elmer, Covina, CA), according to the manufacturer's specifications, in an automatic sequencer ABI PRISM 310 Genetic Analyzer (Perkin Elmer). Sequences obtained were analyzed and deposited in GenBank. Sample sequences were analyzed for homology with other rickettsial species by using the BLASTn program (<http://blast.ncbi.nlm.nih.gov/>).

Serum samples from wild and domestic animals were analyzed for antibodies against antigens of *R. rickettsii*, *R. parkeri*, *R. felis*, *R. belli*, *R. amblyommii*, and *R. rhipicephali* by immunofluorescent assay (IFA). Samples that had titers $\geq 1:64$ were considered positive for *Rickettsia*.

RESULTS

Identification of ticks and fleas obtained from animals in Santa Cruz do Escalvado was performed by using standard taxonomic keys.^{8,9} Species obtained were identified as *Amblyomma cajennense* (n = 96), *Rhipicephalus sanguineus* (n = 55), *Rh. (Boophilus) microplus* (n = 180), *Dermacentor nitens* (n = 11), *Amblyomma brasiliensis* (n = 1), *Ctenocephalides canis* (n = 209), *C. felis* (n = 45), *Xenopsylla cheopis* (n = 3), and *Rhopalopsyllus* sp. (n = 22). Ticks and fleas obtained from dogs, horses, and rodents in Pingo D'Água were identified as *A. cajennense* (n = 35), *Rh. sanguineus* (n = 5), *Rh. (B)*.

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microplus (n = 5), *A. brasiliensis* (n = 5), *A. dubitatum* (n = 1), *C. canis* (n = 24), *C. felis* (n = 26), and *X. cheopis* (n = 11).

Of 42 serum samples from horses and 24 serum samples from dogs obtained in Pingo D'Água and analyzed by IFA against spotted fever group rickettsiae by using antigens from *R. rickettsii*, *R. amblyommii*, *R. rhipicephali*, and *R. belli*, seroreactivity was observed in 16 (38.09%) samples from horses and 2 (8.33%) samples from dogs. In Santa Cruz do Escalvado, of 66 serum samples from horses and 67 serum samples from dogs, seroreactivity was identified in 10 (15.2%) and 14 (20.9%) samples, respectively.

Of 38 serum samples from opossums, 16 showed reactivity against *Rickettsia*-specific antigen and 42.1% of these animals showed reactivity against antigens from *Rickettsia* organisms (Table 1).

In addition to reactivity against *R. rickettsii* antigen, we also observed reactivity against *R. parkeri* (n = 15, 39.5%), *R. amblyommii* (n = 7, 18.4%), and *R. felis* (n = 1, 2.6%). We did not identify seropositive animals by IFA with antigens from *R. belli*.

Rickettsia species DNA was identified in 8 (1.2%) of 646 samples obtained from ectoparasites, anal swab specimens, and blood of captured animals in Santa Cruz do Escalvado. Sequence analysis showed that all positive samples had 100% identity with *R. felis* (CP000053.1).

DISCUSSION

Our results indicate that opossums, horses, and dogs play an important role in the epidemiologic cycle of BSF and other rickettsial diseases in the study areas. Opossums are among the most important genera serving as hosts for fleas and ticks and are useful animals in acarologic studies. At the ecologic level, we observed extensive use of horses for transporting humans and material in urban and rural areas of Santa Cruz do Escalvado and Pingo D'Água. These animals, which can be heavily infested with ticks, can serve as an effective mechanism for dispersal of infected ticks and result in emergence of new disease foci.

The distribution pattern of ectoparasites found in this study, in which there is a predominance of *A. cajennense*, was similar to that of reports from BSF-endemic areas in São Paulo¹⁰ and Minas Gerais.^{2,11,12} These findings indicate that there may be a higher prevalence of this tick species in areas endemic for spotted fever in southeastern Brazil. Dogs, similar to horses, which have easy accessibility to humans, may also play an important role in the epidemiology of BSF, especially if one considers positive serologic results in dogs from areas with high levels of rickettsial infections. Dogs may serve as sentinels for BSF.² Despite low levels of seroreactivity in dogs and

horses in Santa Cruz do Escalvado and the Pingo D'Água, sequencing results for *A. cajennense* obtained from dogs and horses in southeastern Brazil indicate that these ticks harbor a bacteria of the genus *Rickettsia* that needs to be further characterized.

The highest percentage of fleas obtained in this study belonged to the genus *Ctenocephalides*, which is a parasite for dogs, rodents, and opossums. This genus has a worldwide distribution and wide host range and is one of the most common parasites of dogs, cats, and humans. These findings indicate that these fleas may transmit rickettsial infections to domestic and wild animals because of the proximity of their habitats to suburban environments.^{9,13} Although *R. felis* in fleas of the genus *Ctenocephalides* has been observed in areas endemic for spotted fever in São Paulo¹⁴ and Minas Gerais,^{11,15} our findings show a correlation between *R. felis*-infected fleas on dogs and opossums and areas with confirmed cases of BSF.

Additional studies on vector competence and capacity of these arthropods to transmit rickettsial agents to vertebrates are needed to confirm their role in transmission. Absence of a specific diagnostic test for rickettsial disease caused by *R. felis* makes the study of this disease difficult because factors that provide useful data on morbidity and mortality of this disease are absent or indistinguishable from factors for other rickettsiosis in many regions.

There is no commercially available diagnostic test that is species specific for *Rickettsia*. However, results of our study indicate that rickettsiae are currently circulating in Pingo D'Água and Santa Cruz do Escalvado. Although there is no systematic reporting of cases of BSF in these regions, serologic results indicate the presence of spotted fever group rickettsiae in these municipalities. Our findings also indicate the presence of *R. felis* in fleas and ticks and *R. rickettsii* in ticks in Santa Cruz do Escalvado.

Our findings provide useful epidemiologic data on the possible role of more than one species of *Rickettsia* in the etiology of BSF. Serologic and molecular biologic findings suggest the need for establishment and maintenance of effective epidemiological surveillance in the cities studied and in surrounding areas. Additional studies of circulation of emerging diseases in surrounding regions, which are subjected to high anthropogenic effects on the environment, are also needed. Information from these studies may provide useful epidemiologic data and assist in development of strategies to prevent and control these diseases.

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TABLE 1

Reactive samples with titers $\geq 1:64$ by IFA using *Rickettsia*-specific antigens for different animals in Santa Cruz do Escalvado, July 2005–December 2007, and Pingo D'Água, July 2005–May 2006, Minas Gerais, Brazil*

Animal	Municipality	No. tested	No. IFA positive	% Positive
Dogs	Santa Cruz do Escalvado	67	14	21
	Pingo D'Água	24	2	8
Horses	Santa Cruz do Escalvado	66	10	15
	Pingo D'Água	42	16	38
Opossums	Santa Cruz do Escalvado	38	16	42

* IFA = immunofluorescent assay.

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