**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. 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 ≥ 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), *C. canis* (*n* = 209), *C. felis* (*n* = 45), *Xenopsylla cheopis* (*n* = 3), and *Rhipalopsyllus 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).*
micropus (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. rick-
ettsi, R. amblyommii, R. rhipicephali, and R. bell, seroreac-
tivity 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. rickettsi antigen, we also
observed reactivity against R. parkeri (n = 15, 39.5%), R. ambly-
ommii (n = 7, 18.4%), and R. felis (n = 1, 2.6%). We did not iden-
tify seropositive animals by IFA with antigens from R. bell.

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 transport-
ning 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 emerg-
ence 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 Paulo2,3 and
Minas Gerais.1,9,13 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 senti-
nels 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 har-
bor 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 world-
wide 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,1,9,13 our findings shows a correlation between R. felis-
infected fleas on dogs and opossums and areas with con-
firmed 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 rickettsi-
osis 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. rickettsi in ticks in Santa
Cruz do Escalvado.

Our findings provide useful epidemiologic data on the pos-
sible role of more than one species of Rickettsia in the etiol-
ology of BSF. Serologic and molecular biologic findings suggest
the need for establishment and maintenance of effective
epidemiological surveillance in the cities studied and in sur-
rounding 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 epidemi-
ologic data and assist in development of strategies to prevent
and control these diseases.

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