First identification of natural infection of *Rickettsia rickettsii* in the *Rhipicephalus sanguineus* tick, in the State of Rio de Janeiro

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The Brazilian Spotted Fever (BSF) is a zoonotic disease caused by *Rickettsia rickettsii* and transmitted by ticks of the genus *Amblyomma*, more frequently, *Amblyomma cajennense*. The aim of this paper was to report the first molecular detection of *R. rickettsii* on *R. sanguineus* naturally infected in Rio de Janeiro, Brazil. Ticks were collected from dogs in a rural region of Resende municipality, Rio de Janeiro State, Brazil (22°30’9.46”S, 44°42’44.29”W), where occurred five human cases of BSF in 2006. The ticks were identified under a stereoscopic microscope and separated in pools by stages, species and sex. DNA extraction was carried out using QIAamp DNA Mini Kit (QIAGEN®). The DNA was submitted to PCR amplification using 04 set of primers: Rr190.70p/Rr190.602n (*OmpA*, 532bp), BG1-21/BG2-20 (*OmpB*, 650bp), Tz15/Tz16 (17 kDa protein-encoding gene, 246bp) and RpCS.877p/RpCS.1258n (*gltA*, 381bp). PCR products were separated by electrophoresis on 1% agarose gels and visualized under ultraviolet light with ethidium bromide. PCR products of the expected sizes were purified by QIAquick® and sequenced by ABI PRISM®. The generated nucleotide sequences were edited with using Bioedit® software and compared with the corresponding homologous sequences available through GenBank, using Discontiguous Mega Blast (http://www.ncbi.nlm.nih.gov). It was confirmed *R. rickettsii* by sequencing of the material (GenBank FJ356230). The molecular characterization of *R. rickettsii* in the tick *R. sanguineus* emphasizes the role of dogs as carriers of ticks from the environment to home. Moreover, this result suggests that there is a considerable chance for active participation of *R. sanguineus* as one of tick species in the transmission of *R. rickettsii* to human being in the Brazilian territory.

**INDEX TERMS:** *Rhipicephalus sanguineus*, *Acari*, *Ixodidae*, *Rickettsia rickettsii*, molecular analysis, Brazilian spotted fever.

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INTRODUCTION

Brazilian spotted fever (BSF) is a life-threatening rickettsiosis caused by *Rickettsia rickettsii* that is transmitted to human beings by ticks of genus *Amblyomma*, more frequently, *Amblyomma cajennense*, a tick species primarily associated with horses (Dias & Martins, 1939; Oliveira et al. 2000). Although other species of *Rickettsia* have been described in the Brazilian territory, as *A. cajennense*. This tick species is the main vector and reservoir of *Rickettsia conorii* complex that cause Mediterranean spotted fever in Europe, Africa and Asia (Parola et al. 2005).

Although the role of *R. sanguineus* in the transmission cycle of a severe spotted fever rickettsiosis to humans has been demonstrated since 1940’s in Mexico (Bustamante et al. 1946, Bustamante et al. 1947), only in 2002, *R. rickettsii* was identified in *R. sanguineus* in the USA as important vector of the Rocky Mountain Spotted Fever (RMSF) in Eastern Arizona (Demma et al. 2005).

Cardoso et al. 2006, Oliveira et al. 2008), although previous studies had shown evidence of infected *R. rickettsii* in endemic areas in Brazilian states of Minas Gerais and Rio de Janeiro (Lemos et al. 1997a, Rozental et al. 2002). In BSF endemic area, the conglomerations of *R. sanguineus* in endemic areas in Brazilian states of Minas Gerais and Rio de Janeiro (Lemos et al. 1997a, Rozental et al. 2002).

The increasing number of cases of human parasitism by *R. sanguineus* ticks reported in the literature indicates that the interaction between human beings and *R. sanguineus* may be more common than it is recognized in the American continent.

Usually, *R. sanguineus* parasites dogs in urban areas, but sometimes they are found in rural farming areas (Soares et al. 2006, Labruna et al. 2001). In BSF endemic area, the conglomerations of *R. sanguineus* create a great risk to humans whose can be attacked by infected ticks in and around their houses. This study reports the first molecular detection of *R. rickettsii* in *R. sanguineus* ticks in Rio de Janeiro State, Brazil.

MATERIALS AND METHODS

The material here analyzed is part of an ongoing study that is being developed at Engenheiro Passos District (22°30’165’S, 44°42’654’W), located in the city of Resende, Rio de Janeiro State, Brazil where in May 2006 five BSF cases with two deaths were confirmed by serology. This area is situated in the western portion of the State of Paraiba do Sul middle river valley. The native vegetation, Atlantic Forest, has been replaced by
RESULTS AND DISCUSSION

From the 1.445 ticks collected, Rhipicephalus sanguineus, Amblyomma cajennense, A. aureolatum, A. ovale, Dermacentor (Anocentor) nitens and Rhipicephalus (Boophilus) microplus, besides immature stages of Amblyomma sp. from animals, only 22 were submitted to molecular analysis until now. The result revealed PCR products for one specimen of R. sanguineus which showed amplicons to the four set of primers with 99% similarity to R. rickettsii (GenBank FJ356230). The infected tick was identified as an engorged female, collected directly from a male dog, approximately one-year old, of Poodle breed, who lived freely in different environments in the rural property where the BSF cases occurred.

The occurrence of five confirmed cases of BSF in this region and the lack of A. cajennense infected by R. rickettsii, until now, is in agreement with the data showed by Demma et al. (2005) in which R. rickettsii was detected in R. sanguineus in an area in Arizona where the main vectors in United States, D. variabilis and D. andersoni were not identified.

More publications have showed evidence that the brown dog tick is the RMSF vector in some regions of the USA, where close human interaction with free-roaming community dogs heavily parasitized by these ticks is observed. Also, in California, where the role of R. sanguineus is unclear as a potential vector of this disease, R. rickettsii was detected in this tick, but some differences in genotypes were shown between the strains isolated from Arizona, demonstrating a significant genetic diversity in this species of the spotted fever group rickettsiae SFGR (Wikswo et al. 2007).

Although until now there is not strong evidence that R. sanguineus as the vector of BSF, previous studies have shown evidence of involvement of R. sanguineus in endemic regions in the states of Minas Gerais and Rio de Janeiro. Hemolymph samples of specimens collected from R. sanguineus were analyzed by Giemsa stain and immunofluorescence assay and microorganisms with similar morphology to (SFGR) were detected (Lemos et al. 1996, Lemos et al. 1997a, Rozental et al. 2002).

Currently, in Brazil, the main tick implicated in epidemiology of the BSF is A. cajennense, an ixodid with low specificity for the vertebrate host, its immature stages commonly are found parasitizing human beings in rural areas or at least where primary hosts are found (Lemos et al. 1997b, Oliveira et al. 2000). A. aureolatum is another involved tick in the epidemiology of BSF, feeding on wild animals during it immature stages and parasitizing dogs during the adult stage. R. rickettsii was identified in this tick, collected from dogs at an endemic region of São Paulo state, suggesting be an important vector in Brazil (Pinter & Labruna 2006).

Dogs from rural areas move freely in pastures, forests and stables around the houses, most of them being pets, mainly for children. Thus, the presence of these animals in homes is a risk factor for the occurrence of human cases, because they carry ticks from their natural environment into the house and/or to the neighboring areas.

Although this result is preliminary, the presence of R. sanguineus infected by R. rickettsii in a BSF endemic area confirms that this species may play a role in transmitting SFGR to human in Resende, state of the Rio de Janeiro, Brazil, an area where dogs, horses, cattle; domestic cats and their ticks have continuous contact with wild animals such as capybaras, marsupials and small rodents.

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REFERENCES


