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Rickettsia spp. infection in *Rhipicephalus sanguineus* ticks in a Brazilian spotted fever endemic rural area in Rio de Janeiro state, Brazil

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INTRODUCTION

Brazilian spotted fever (BSF) is a life-threatening zoonotic tick-borne disease, caused by *Rickettsia rickettsii*, which is transmitted to humans through the bite of infected ticks. BSF is the most prevalent rickettsial disease in Brazil. *Amblyomma cajennense* ticks are considered as being its main vector and reservoir. The immature stage of *A. cajennense* is usually a parasite on humans and is considered to be an eclectic tick, because it feeds on different animal species. *Rhipicephalus sanguineus*, although considered as a vector of *R. rickettsii* in eastern Arizona and Mexico, is not the common vector for BSF [1]. In Brazil, *R. sanguineus* is usually found on dogs from urban and rural environments. BSF is described in several regions of southern Brazil, mainly in the states of Minas Gerais, Rio de Janeiro and São Paulo [2]. In Rio de Janeiro state, since 1970, suspected and confirmed cases of BSF have been detected. Barra do Pirai is a city belonging to the Rio de Janeiro state and 154 km away from the capital. It has been considered to be an endemic area of BSF since 2004, when our group characterized *R. rickettsii* from a human fatal case [3]. In this report, the authors investigate the presence of *Rickettsia* spp. in ticks collected in this area by molecular analysis and show their possible implication as a vector of BSF in this endemic area.

MATERIAL AND METHODS

During the years 2002 and 2003, after notification of a young woman's death from an infectious disease compatible with BSF, a study was carried out. Ticks were collected from vegetation and dogs from 10 different localities in Barra do Piraí. *Rhipicephalus sanguineus* was the most abundant collected species. The ectoparasites were identified through a stereoscopic microscope using Aragão and Fonseca's taxonomic keys for ticks [4]. Weeks after, a male house servant presented with a similar infectious disease, which evolved to death. He was submitted to necropsy and immunohistochemical staining suggested a spotted fever group rickettsiae and sequence analysis showed identity to *R. rickettsii* [3].

DNA was extracted from individual ticks by freezing them in liquid nitrogen and crushing with a sterile micropestle, resuspended with 20 μL of sterile brain heart infusion (BHI) and stored at −20°C until nucleic acid extraction. Total DNA was extracted from the pulverised ticks using QIAamp DNA Mini Kit (QIAGEN™ Hilden, Germany). The DNA was divided into pools organised by locality, species and sex and screened for the presence of *Rickettsia* DNA by PCR using four sets of primers: Rr190.70p/Rr190.602n (OmpA – 532 bp), BG1-21/BG2-20 (OmpB – 650 bp), Tz15/Tz16 (17 kDa – 246 bp) and RpCS.877p/RpCS.1258n (gltA – 381 bp) [5]. If a pool demonstrated an expected PCR product, DNA of each tick specimen that made part of that pool was individually tested.

PCR conditions consisted of an initial DNA denaturation and hot start at 95°C for 5 min, followed by 40 consecutive cycles of 40 s denaturation at 95°C, primer annealing at 55°C for 1 min, extension at 72°C for 1 min 10 sec, and a 7-min extension at 72°C. For each reaction, 8 μL of the DNA template from each individual tick sample were added to 2.5 μL PCR buffer (10× Invitrogen™, Carlsbad, CA, USA), 1.2 μL of each primer (20 mM), 1.5 μL $MgCl_2$ (3 mM), 0.25 μL of dNTP mixture (20 mM), 0.25 μL Platinum Taq DNA Polymerase (5 U/μL Invitrogen™) and nuclease free water to a final volume of 25 μL. A total of 5 μL of DNA extracted from *R. rickettsii*-infected *A. cajennense* ticks was used as positive control. PCR products were stained by ethidium bromide and visualised by electrophoresis in 1% agarose gel.

RESULTS

A total of 1233 ticks were collected; 1017 belonged to *R. sanguineus* species, 1 to *A. aureolatum*, and 215 belonged to *Amblyomma* genus. Due to the viability of the samples, only 259 ticks were tested and divided into 52 pools. Thirty-six pools were positive and when individually tested showed expected bands in 85 ticks to OmpB and 17kDa. Eleven ticks showed expected bands to both primers (Table 1).

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Table 1. PCR results for the tick species and stages of development collected from vegetation and dogs in Barra do Piraí, State of Rio de Janeiro, Brazil

Species	Stage	No. of ticks analysed	Positive ticks with		
			OmpB primer only/number analysed (%)	17 kDa primer only/number analysed (%)	Both primers/number analysed (%)
Amblyomma sp.	Nymph	78	02 (2.56)	0 (0.00)	0 (0.00)
Amblyomma cajennense	Male	01	0 (0.00)	0 (0.00)	0 (0.00)
R. sanguineus	Male	47	15 (31.92)	03 (6.4)	03 (6.4)
R. sanguineus	Female	133	43 (32.33)	11 (8.3)	08 (6.02)
TOTAL		259	60 (23.2)	14 (5.41)	11 (4.25)

CONCLUSIONS

Although the DNA sequences from this study have not yet been characterized, the identification of 85 PCR-positive ticks confirms the participation of *R. sanguineus* as a possible vector of BSF in this endemic area and reinforces the importance of domestic dogs as potential infection amplifiers.

It is very important to notice that, in the same area, recently, our group characterized *R. rickettsii* in *R. sanguineus* ticks (Cunha NC, Fonseca AH, Rezende J, Rozenthal T, Favacho ARM, Barreira JD, Massard CL, Lemos ERS).

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