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Rhipicephalus (Boophilus) microplus: Rickettsiae infection in Brazil

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ABSTRACT

The tick *Rhipicephalus (Boophilus) microplus* has been considered a major pest of cattle in tropical regions of the world, inducing significant economic impact on livestock activity. In Brazil, the scenarios of Spotted Fever (SF) epidemic cycles involve *Amblyomma sculptum* and *Amblyomma aureolatum*, transmitting *Rickettsia rickettsii*, as well as *Amblyomma ovale*, transmitting *R. parkeri* strain Atlantic Rainforest. However, other potentially pathogenic species of *Rickettsia*, as well as new species of ticks with vector potential, have been reported for Brazil. Thus, during SF focus investigation and environmental surveillance performed by Brazilian National Network of Environment Surveillance for Tick-borne Diseases between 2011 to 2017, ectoparasites were collected from vertebrate hosts and the environment in rural and urban areas of different Brazilian biomes. Here we analysed Rickettsiae infection in *Rh. microplus* and possible roles of this tick species in the maintenance and circulation of *Rickettsia* sp. in SF endemic areas. Cattle tick samples were naturally infected with *R. rickettsii*, *R. parkeri*, *R. felis*, *R. tamurae*, *R. rhipicephali* and *R. bellii*. The data in this study demonstrate that *Rh. microplus* acquires *Rickettsia* infection, including pathogenic species, and indicates this tick as suggested marker of Rickettsiae potential presence or circulation.

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Introduction

Rhipicephalus (Boophilus) microplus, also known as the cattle tick, has a broad geographic distribution, being found mainly in tropical and subtropical regions (Estrada-Peña et al. 2006). Although *Rh. microplus* demonstrates a high degree of host-specificity and requires only one host to complete its lifecycle (on which it performs all the blood meals), *Rh. microplus* can parasitize alternative hosts, including humans (Soares et al. 2007).

The main importance of *Rh. microplus* is related to its significant economic impact on livestock. In Brazil, the cattle tick is distributed throughout the country and it is estimated that 80% of the national cattle herd is infested, causing an economic loss of three billion dollars a year. Under such circumstances, direct spoilage and the participation in the epizootic cycle of different pathogens (e.g. *Babesia bovis*, *B. bigemina*, *Anaplasma marginale*) are the main ways in which these ticks interact with disease development and deleterious influence on domestic bovines (Peter et al. 2005; de la Fuente et al. 2008; Grisi et al. 2014).

Due to its wide distribution in the country, *Rh. microplus* is found in Spotted Fever (SF) endemic regions. However, even though it has been reported as being infected by different species of Spotted Fever Group Rickettsiae (SFGR) (Bermúdez et al. 2009; Moura-Martiniano et al. 2014; Pesquera et al. 2015), the involvement of this tick in transmitting these bacteria remains incipient (Cordeiro et al. 2018).

In Brazil, known SF epidemic cycle scenarios involve *Amblyomma sculptum* and *A. aureolatum* transmitting *R. rickettsia*, and *A. ovale* transmitting *R. parkeri* strain Atlantic Rainforest (Szabó et al. 2013; Oliveira et al. 2016a; Nieri-Bastos et al. 2018). However, SF human cases recorded in new outbreaks in Brazil indicate clinical profiles that differ from those already established. Without already recognized vectors or detection of *Rickettsia* in these vectors, it is difficult to characterize the bioagent circulation mechanism involved in such

outbreaks (e.g. Oliveira et al. 2016a, 2016b, 2017, 2018). In addition, in recent decades, other potentially pathogenic species of *Rickettsia*, as well as new species of ticks with vector potential, have been reported from Brazil (e.g. Labruna et al. 2011; Nieri-Bastos et al. 2014; Nunes et al. 2015; Moerbeck et al. 2016; Weck et al. 2016; Silva et al. 2017a, 2018), indicating SF cycle complexity and the existence of various distinct ecoepidemiological scenarios within the national territory. Furthermore, doubts still remain concerning the role of *Rh. microplus* in Rickettsiae circulation.

Here, we aim to investigate and molecularly characterize the presence of *Rickettsia* sp. in *Rh. microplus* specimens from areas of different ecological features in Brazil.

Materials and methods

During SF focus investigation and environmental surveillance performed between 2011 to 2017, State and Municipal Health Secretaries collected ectoparasites from vertebrate hosts and the environment (Figure 1) in rural and urban areas (pasture, farms and river bank) across a variety of Brazilian regions (Supplementary Table 1).

After morphological identification of 7,418 specimens of *Rh. microplus* (Aragão and Fonseca 1961; Marquez et al. 1992; Barros-Battesti et al. 2006), a total of 801 specimens were analysed individually (nymph and adults ticks) or by pools of 10 larvae, comprising 620 samples.

These samples were initially snap-frozen in liquid nitrogen for genomic DNA (DNAg) extraction, performed following the saturated saline solution protocol (Aljanabi and Martinez 1997), quantified by spectrophotometry (NanoDrop™ 2000, Thermo Scientific™) and used as template for PCR screenings for rickettsial genes *gltA* (CS-78/CS-323, Labruna et al. 2004a), *ompA* (Rr190.70p/Rr190.602n, Regnery et al. 1991), *sca4* (D1738F/D2482R, Sekeyova et al. 2001),

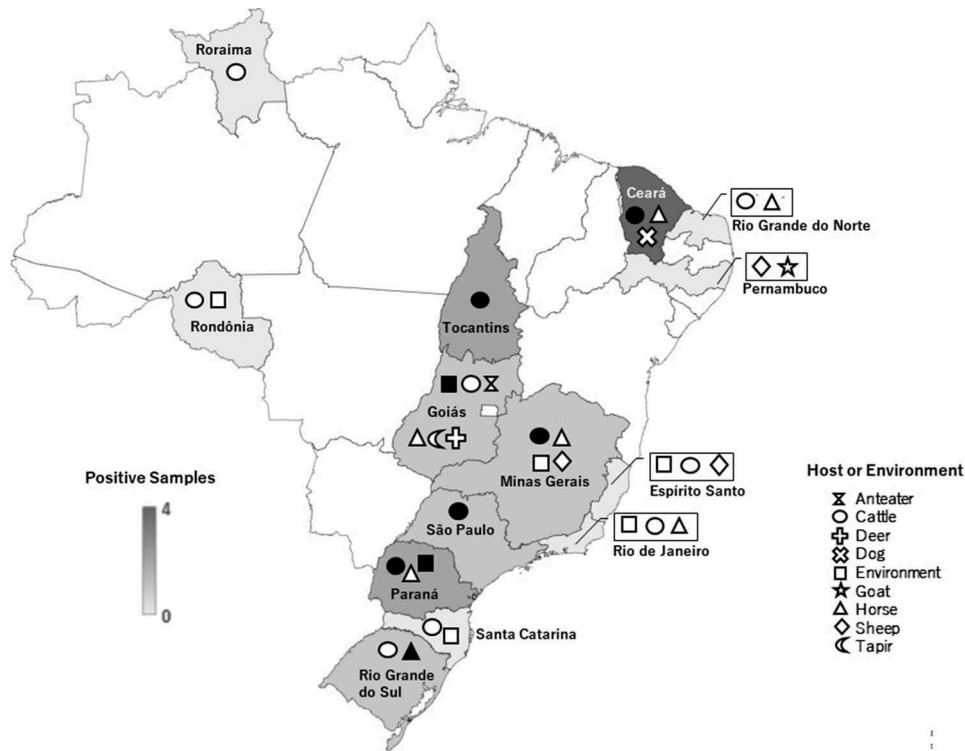


Figure 1. Geographical distribution and host/environment of *Rhipicephalus (Boophilus) microplus* specimens collected by federative unit and Rickettsiae-positive samples, during Spotted Fever focus investigation and environmental surveillance performed in Brazil from 2011 to 2017. White filled forms indicate host or environment from tick collected and black filled forms indicate host or environment from PCR-positive tick samples.

htrA (17k-3/17k-5, Labruna et al. 2004b) and *ompB* (120.M59/120.807, Roux and Raoult 2000), as well as mitochondrial *cytochrome oxidase I* (COI) gene (COX-1.2F/COX-1.2R, Csordas et al. 2016) and 12S ribosomal DNA (T1B/T2A, Beati and Keirans 2001) tick molecular markers. Analyses included DNA-free reactions as negative controls and 300 ng of *R. rickettsii* DNAG as positive controls of rickettsial amplification reactions. Amplified DNA products were electrophoresed through a 2% agarose gel, stained with ethidium bromide, and examined by UV transillumination.

PCR products of the expected size were purified using Wizard® SV Gel and PCR Clean-Up System (Promega™), sequenced applying the BigDye™ Terminator–Cycle Sequencing Ready Reaction kit (Applied Biosystems™) and analysed in an automated ABI 3730xl DNA analyser (Applied Biosystems™). DNA sequences were edited with ChromasPro 1.5 software (Technelysium Pty Ltd), with identity values obtained by BLAST analysis (<http://blast.ncbi.nlm.nih.gov>). A Maximum-Likelihood tree was inferred using PhyML 3.0 online software (Guindon et al. 2010) with GTR+G correction model, selected by Smart Model Selection (Lefor et al. 2017). The reliability of the tree topology was evaluated via bootstrap support (1000 repeats).

Results

A total of 12 *Rh. microplus* samples collected from horses, cattle and the environment were positive for rickettsial genes (Figure 1). BLAST analysis of the obtained sequences revealed high nucleotide-identity rates with *R. rickettsii* (MH194350, MH194352), *R. belli* (MH194351), *R. parkeri* strain Atlantic rainforest (MK720994, MK720995, MH194354, MH194355, MH194358), *R. felis* (MK720997, MH194353, MH194356, MH194360), *R. rhipicephali* (MK720996, MK720998) and *R. tamurae* (MK720993), totalling 15 obtained sequences (Table 1).

To confirm tick identification, we analysed the sequences of 12S rDNA (MN081899) and COI genes (MN088852) amplified from randomly chosen adult (sample code LIC 3764A) and pooled larval samples (sample code LIC 7156), and BLAST analysis revealed 100% identity sequences (380/380 for 12S and 645/645 for COI)

with sequences of *Rh. microplus* (KP143546 and KC503261) deposited in GenBank.

In addition, phylogenetic inferences were performed with concatenated sequences of *gltA*, *ompA*, *ompB*, *htrA* and *sca4* genes, using sequences corresponding to a sample of each Rickettsiae identity, and the resulting tree grouped: sequences from Ceará State with *R. rickettsia*, *R. tamurae* and *R. bellii* clusters; sequences from São Paulo State with *R. rhipicephali* cluster; sequences from Paraná State with *R. parkeri* strain Atlantic rainforest (identified in Barbieri et al. 2014 as strain Aa46) cluster; and sequences from Rio Grande do Sul State with *R. felis* cluster (Figure 2).

Due to the obtained sequence sizes, bootstrap values were low in some tree clusters, indicating similar portions in analysed species fragments. However, the observed BLAST values mostly showed 100% overlap with the respective Rickettsiae sequence, and of the total sequences, one *ompB* sequence showed 98% similarity with *R. felis* and two *gltA* sequences showed 99% similarity with *R. rhipicephali* and with *R. bellii* (Table 1).

Discussion

Studies to date have detected *Rh. microplus* infected with *R. amblyommatis* in Panama (Eremeeva et al. 2009), *Rickettsia* sp. strain Columbianense in Colombia (Miranda et al. 2012), *Rickettsia* sp. strain 12G1 in Ecuador (Pesquera et al. 2015), and *R. rickettsii* in Brazil (Moura-Martiniano et al. 2014). Our results show for the first time, sequences genetically related to *R. tamurae*, *R. rhipicephali*, *R. parkeri*, *R. felis* and *R. bellii* in *Rh. microplus* and also sequences closely related to *R. rickettsii* in *Rh. microplus* for the first time in Ceará state, northeastern Brazil.

The Columbianense and 12G1 Rickettsiae strains are genetically related to *R. tamurae*, as well as other strains recorded in Brazil: e.g. strain Pampulha from Minas Gerais state (Guedes et al. 2011; Szabó et al. 2013), strain Serra dos Órgãos from Rio de Janeiro state (Spolidorio et al. 2012), and strain Aragoai from Paraná State (Blanco et al. 2016). Although there is evidence of this *Rickettsia* occurring in different areas of Brazil and in different vector species,

in an enzootic cycle associated with *A. ovale* and dogs (Szabó et al. 2013; Moerbeck et al. 2016; Vizzoni et al. 2016; Acosta et al. 2018). The capacity of *Rh. microplus* to transmit *R. parkeri* is experimentally inconclusive (Cordeiro et al. 2018) and, considering the ecological scenario of this bioagent's cycle, may have no epidemiological importance.

Cattle tick acquisition of pathogenic *Rickettsia* may also occur by a co-feeding process, already established for Rickettsiae (Socolovschi et al. 2009). *Rickettsia parkeri* has been reported from *Amblyomma triste*, an Ixodid tick with the capacity to parasitize several vertebrates, including cervids and bovines (Labruna et al. 2003; Mertins et al. 2010), host species that share the same environments, allowing infection of *Rh. microplus* by *R. parkeri*.

Additionally, here we also report for the first time *R. felis* in cattle ticks, a bacterium with wide geographical distribution, including all Brazilian regions (Horta et al. 2014). However, besides being detected in various species of blood-feeding arthropods, the majority of reports are associated with the flea genus *Ctenocephalides* (Reif and Macaluso 2009; Parola 2011; Mediannikov et al. 2012), common worldwide as ectoparasites of various carnivores, especially dogs. Thus, this Rickettsiae detection in other species of dog ectoparasites seems not to be uncommon (Gehrke et al. 2009; Parola et al. 2013). However, reports, from various states of Brazil of its presence in *Rh. microplus* (Table 1) are important, especially considering that cattle ticks do not commonly parasitize carnivores. The specimens found in this study infected with *R. felis* were collected from cattle, horses and the environment (pasture), without evidence of the infection mechanisms.

Other sequences from Ceará state, northeastern Brazilian, were identified as *R. rickettsii* and *R. bellii*. The samples infected by *R. rickettsii* were collected in Maciço do Baturité, an SF endemic area (Moerbeck et al. 2016), with high-altitude forest, with conditions of high humidity and mild temperature conditions within the otherwise semiarid climate of the Caatinga biome. Although SF cases reported in the region were associated with *R. parkeri* (= *Rickettsia* sp. strain Atlantic rainforest) transmitted by *A. ovale* (Moerbeck et al. 2016), and despite having no reports of SF cases clinically associated with *R. rickettsii*, this species was also isolated from *Rh. sanguineus* collected on a dog from Maciço do Baturité (Silva et al. 2017b). Together with the results here obtained, this detection indicates the circulation of *R. rickettsii* in the region, and in a variety of tick species. Thus, due to the public health importance of *R. rickettsii*, it is necessary to understand the enzootic cycle throughout the region, in order to understand the potential epidemiological scenario and take preventive measures to prevent new occurrences of *R. rickettsii* SF in Brazil. In this context, *Rh. microplus* becomes even more important as an object of study, since its potential for *R. rickettsii* transmission has been experimentally demonstrated (Monteiro and Fonseca 1932).

Besides pathogenic Rickettsiae, the generally considered non-pathogenic *R. bellii* is a widely distributed species, having been detected from a great variety of hosts, indicating a broad circulation capacity (Labruna et al. 2004a; Tomassone et al. 2010; Parola et al. 2013) and possibly playing an important role in the ecology and epidemiology of other Rickettsiae species (Macaluso et al. 2002). The first record of this species in Maciço do Baturité was in an *A. nodosum* tick collected from a wild animal (Moerbeck et al. 2016). Here we presented the first report for Brazil of *R. bellii* detected in *Rh. microplus*, indicating the circulation of this bacterium throughout the studied region.

It is known that a primary infection with one species of *Rickettsia* would prevent transovarian transmission of a second *Rickettsia* species (Burgdorfer 1988; Macaluso et al. 2002). This was recently corroborated by an experimental study conducted with *A. dubitatum*, where primary *R. bellii* infection appeared to decrease the effectiveness of subsequent *R. rickettsii* transovarian transmission (Sakai et al. 2014).

Conclusion

In spite of the wide distribution of *R. microplus* throughout Brazil, because this species has rarely been reported on humans, the participation of the cattle tick in Rickettsiae enzootic and epidemic cycles remains unknown. The data from the current study demonstrate that *Rh. microplus* can naturally acquire *Rickettsia* infection, including infection with pathogenic species, and suggests that this tick could serve as an indicator of Rickettsiae presence or circulation.

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Disclosure statement

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