

First molecular detection of *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma ovale* ticks from Espírito Santo state, Brazil

Primeira detecção molecular da *Rickettsia* sp. cepa Mata Atlântica em carrapatos *Amblyomma ovale* do estado do Espírito Santo, Brasil

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Abstract

Espírito Santo state (southeastern Brazil) is considered an endemic area for spotted fever group rickettsioses. In February 2017, we received in our laboratory seven unfed *Amblyomma ovale* adult ticks collected by a farmer from his clothes and body (not attached) during a working day in the rural area of Ibiraçu municipality, Espírito Santo state. By polymerase chain reaction (PCR) analyses, targeting *gltA* and *ompA* rickettsial genes, the DNA of *Rickettsia* was detected in 6/7 (85.7%) *A. ovale*. In all cases, DNA sequencing of PCR products revealed that consensus sequences of both genes were 100% identical to *gltA* and *ompA* corresponding sequences of *Rickettsia* sp. strain Atlantic rainforest retrieved from GenBank. This study reports the first molecular detection of *Rickettsia* sp. strain Atlantic rainforest in *A. ovale* ticks from Espírito Santo state. Our findings indicate a new Brazilian state in the southeast region at risk of human infection with this tick-borne emerging rickettsial agent.

Keywords: *Rickettsia* sp., strain Atlantic rainforest, *Amblyomma ovale*, ticks, Espírito Santo, Brazil.

Resumo

O estado do Espírito Santo (Sudeste do Brasil) é considerado área endêmica para riquetsioses do Grupo Febre Maculosa. Em fevereiro de 2017, recebemos em nosso laboratório sete carrapatos adultos *Amblyomma ovale* não ingurgitados, coletados por um fazendeiro nas suas roupas e corpo (não fixadas) durante um dia de trabalho, em área rural do município de Ibiraçu, estado do Espírito Santo. Por meio de reação em cadeia da polimerase (PCR), amplificando os genes riquetsiais *gltA* e *ompA*, foi detectado ADN de *Rickettsia* em 6/7 (85,7%) dos *A. ovale*. O sequenciamento dos produtos de PCR indicou que as sequências consenso de ambos genes foram 100% idênticos às sequências correspondentes dos genes *gltA* e *ompA* da *Rickettsia* sp. cepa Mata Atlântica recuperadas do GenBank. Este estudo relata a primeira detecção molecular da *Rickettsia* sp. cepa Mata Atlântica em carrapatos *A. ovale* do estado do Espírito Santo. Nossos resultados apontam um novo estado brasileiro da região Sudeste com risco de infecção humana por este agente rickettsial emergente transmitido por carrapatos.

Palavras-chave: *Rickettsia* sp., cepa Mata Atlântica, *Amblyomma ovale*, carrapatos, Espírito Santo, Brasil.

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Spotted fever group (SFG) rickettsioses are currently recognized as emerging or re-emerging zoonotic diseases, caused by pathogenic bacteria of the genus *Rickettsia* and transmitted to human beings through tick bites (PAROLA et al., 2013). In Brazil, since the 1920s, *Rickettsia rickettsii* has been recognized as the main etiological agent of SFG rickettsioses, associated with *Amblyomma sculptum* (formerly *Amblyomma cajennense*) and *Amblyomma aureolatum* ticks as vectors, causing a highly lethal illness, which has been predominant by far in the southeast Brazilian region (OLIVEIRA et al., 2016). Nevertheless, in 2010, a Brazilian case of mild eschar-associated rickettsiosis was reported from the littoral region of São Paulo state, and the molecular characterization of its etiological agent revealed another SFG agent, which was named the *Rickettsia* sp. strain Atlantic rainforest (SPOLIDORIO et al., 2010b). After this report, new human cases related to *Rickettsia* sp. Atlantic rainforest have been documented from Brazilian patients from Bahia and Santa Catarina states (SILVA et al., 2011; KRAWCZAK et al., 2016c). Research to unveil its epidemiology has identified the tick *Amblyomma ovale* as its main vector (KRAWCZAK et al., 2016a). The results based on experimental models and field-collected material indicate that this tick species is naturally infected with strain Atlantic rainforest in several states of the south (Rio Grande do Sul, Santa Catarina and Paraná), southeast (São Paulo and Rio de Janeiro) and northeast (Bahia and Ceará) regions of Brazil (KRAWCZAK et al., 2016b; NIERI-BASTOS et al., 2016; MOERBECK et al., 2016; LUZ et al., 2017).

Espírito Santo state (southeastern Brazil) is considered an endemic area for SFG rickettsioses (OLIVEIRA et al., 2016). Previously published reports, based on serological, immunohistochemical, and epidemiological methods, have suggested *R. rickettsii* as the probable etiological agent of an outbreak in the 1990s (COREY et al., 1993; SPOLIDORIO et al., 2010a). Consequently, we recently confirmed *R. rickettsii* as the etiological agent of fatal SFG rickettsiosis in human patients from Espírito Santo state between 2015 and 2017 (FACCINI-MARTÍNEZ et al., 2018). The aim to determine the identity of other pathogenic SFG rickettsiae circulating in Espírito Santo prompted the current study.

In February 2017, we received in our laboratory seven unfed adult ticks (stored in a plastic vial with 70% ethanol) that were collected by a farmer from his clothes and body (not attached) during a working day in the rural area of Ibirapu municipality, Espírito Santo state (Figure 1). Ticks were identified following a taxonomic key for the *Amblyomma* genus (BARROS-BATTESTI et al., 2006) and tested for rickettsial DNA. For this purpose, ticks were processed individually for DNA extraction using the guanidine isothiocyanate phenol technique (SANGIONI et al., 2005). Then, the obtained material was tested by the polymerase chain reaction (PCR) technique using primers CS-78 and CS-323 targeting a 401-bp fragment of the citrate synthase gene (*gltA*), which is common in all representatives of the genus *Rickettsia* (LABRUNA et al., 2004). Samples yielding expected size amplicons were subsequently tested with primers Rr190.70p and Rr190.701n, targeting a 631-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*) (EREMEEVA et al., 2006). The negative control tube containing ultrapure water and a positive control tube containing DNA of the *Rickettsia* sp. strain NOD were included in each PCR run. The obtained amplicons were purified with ExoSap (USB,

Cleveland, Ohio, USA) and DNA-sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used for PCR. The obtained sequences were assembled with Geneious R9 software and submitted to Blast analyses (NCBI, 2017) to infer the closest similarities available in GenBank.

All seven adult ticks were morphologically identified as *A. ovale* (4 males and 3 females). Six ticks (85.7%) were found to contain rickettsial DNA by PCR protocols targeting *gltA* and *ompA* genes. The obtained partial sequences of the *gltA* gene (350-bp) were identical to each other, and identical sequences of *ompA* gene (590-bp) were obtained for all ticks as well. Consensus sequences of both genes were 100% identical to *gltA* and *ompA* corresponding sequences of *Rickettsia* sp. strain Atlantic rainforest (GenBank accession numbers KJ174527 and KJ158741, respectively). The DNA sequences generated in the present study have been submitted to GenBank (accession numbers MF536974, MF536975).

The present study reports for the first time the detection and molecular characterization of the *Rickettsia* sp. strain Atlantic rainforest in *A. ovale* ticks in the Espírito Santo state. Interestingly, the ticks tested in our study were collected from the clothes and body of a farmer, and although they were not found attached to his skin, our findings indicate an eventual risk for a possible tick bite and related pathogenic *Rickettsia* transmission. This is because *A. ovale* is recognized as both an anthropophilic tick in South America (GUGLIELMONE et al., 2006) and a competent vector for *Rickettsia* sp. strain Atlantic rainforest (KRAWCZAK et al., 2016a). Furthermore, our data identified an additional location (Ibirapu municipality) in the state of Espírito Santo where *A. ovale* was present. In a recent study on tick fauna in the same state, *A. ovale* was only documented for the municipalities of Santa Teresa and Santa Maria de Jetibá (ACOSTA et al., 2016). Nevertheless, it is quite likely that its distribution would be even broader in Espírito

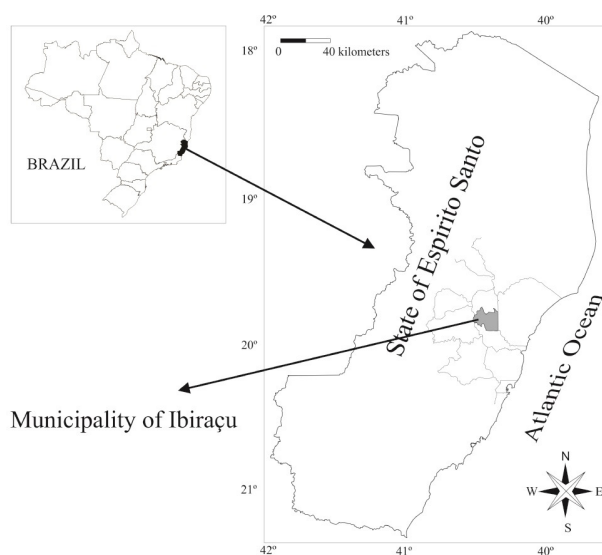


Figure 1. Map of Ibirapu municipality in Espírito Santo state, Brazil.

Santo since this state has an important portion of the preserved Atlantic rainforest.

Finally, our findings note a new Brazilian state within the southeast region in which the risk of human infections with *Rickettsia* sp. strain Atlantic rainforest, an agent of an emerging tick-borne human disease, exist. A recent study provided phylogenetic evidence that the *Rickettsia* sp. strain Atlantic rainforest represents a genetic variant of the human pathogen *Rickettsia parkeri* (PADDOCK et al., 2017). In this way, it would be important to conduct an active epidemiological search with the purpose of characterizing new eschar-associated rickettsiosis cases in Brazil.

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