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ORIGINAL ARTICLE

DETECTION OF A *Rickettsia* GENOTYPE RELATED TO THE OLD WORLD INFECTING *Amblyomma sculptum* TICK IN AN ENDEMIC AREA OF BRAZILIAN SPOTTED FEVER

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ABSTRACT

Brazilian Spotted Fever is an important tick-borne disease caused by *Rickettsia rickettsii* and transmitted mainly by the human-biting tick *Amblyomma sculptum*. During an epidemiological surveillance in Pedro Leopoldo, an endemic area of Minas Gerais State, southeastern Brazil, ectoparasites were collected from vertebrate hosts and from the environment. Rickettsial genes were obtained from a male *A. sculptum* and the resulting phylogenetic tree grouped this bacterium with *Rickettsia* sp. isolate Pampulha, a strain closely related to the pathogenic species *Rickettsia tamurae* and *Rickettsia monacensis*. This is the first report of sequences phylogenetically related to *R. tamurae* and *R. monacensis* infecting *A. sculptum* in Brazil.

KEY WORDS: Spotted fever group *Rickettsia; Amblyomma sculptum*; spotted fever focus; Ixodidae; Brazil.

INTRODUCTION

Rickettsiosis are re-emerging zoonosis, caused by gram-negative and intracellular species of the genus *Rickettsia* (Parola et al., 2013) occurring worldwide. In Brazil, the main Rickettsiosis is Brazilian Spotted Fever (BSF), a severe disease with confirmed cases from all regions of the country (Oliveira et al., 2016a; Oliveira et al. 2016b). The Southeastern region of Brazil has the highest number of BSF confirmed cases and the most deaths, where the eco-

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epidemiological scenario involves capybaras and horses as the vertebrate hosts, and the human-biting tick *Amblyomma sculptum*, which is the main vector of *Rickettsia rickettsii*, the etiologic agent of BSF (Oliveira et al., 2016a).

Meanwhile, studies of rickettsial diseases in BSF endemic and non-endemic regions have enhanced knowledge of pathogenic and pathogenicity-unknown rickettsiae of the Spotted Fever Group (SFG) (Szabó et al., 2013; Oliveira et al., 2016a), increasing the number of new species known to be circulating in the country, including strains related to *Rickettsia tamurae* and *Rickettsia monacensis*. These SFG rickettsiae are often reported in human cases from Asia and Europe and, recently, strains of *Rickettsia* phylogenetically related to both of them have been detected in tick samples from Minas Gerais, Rio de Janeiro and Paraná states, known as strains Pampulha (Almeida et al., 2011), Serra dos Órgãos (Spolidorio et al., 2012) and Aragaoi (Blanco et al., 2016), respectively.

Here, we evaluate rickettsial infection in ticks and fleas collected from vertebrate hosts and from the environment in Minas Gerais, one of the three Brazilian states with the greatest number of confirmed BSF cases and deaths (Oliveira et al., 2016a).

METHODS

In December 2012, during an epidemiologic surveillance performed by the Brazilian Network of Environment Surveillance for Tick-borne Diseases, in a BSF endemic area, in the municipality of Pedro Leopoldo, Metropolitan Region of Belo Horizonte, in a Cerrado biome sector of Minas Gerais State, Southeast Brazil, a total of 1,003 ticks and 21 fleas were collected from six dogs, four cows, six horses and three sheep (totaling 19 examined vertebrates) and from the environment (Table). Ectoparasites were removed from the vertebrate hosts from the Sustainable Production Center of Pedro Leopoldo Model Farm at the Federal University of Minas Gerais (coordinates: 19°38´06.07´´S 44°03´09.22´´W), and by flannel dragging in the pasture areas, then were morphologically identified (Aragão & Fonseca 1961; Amorim et al., 1997; Marquez et al., 1992; Linardi & Guimarães, 2000; Barros-Battesti et al., 2006) and individually submitted to DNA extraction (Aljanabi et al., 1997). Polimerase Chain Reaction (PCR) screening for rickettsial infection was performed with primers for *OmpA* (Rr190.70p/Rr190.602n) (Regnery et al., 1991), *OmpB* (120-2788/120-3599) (Roux and Raoult 2000) *Sca4* (D1738F/D2482R) (Sekeyova et al., 2001), *gltA* (CS-78/CS-323 and CS-239/CS-1069) (Labruna et al., 2004a; Labruna et al., 2004b) and *htrA* (17k-3/17k-5) (Labruna et al., 2004a) genes, using Molecular Grade Water as negative control and 300ng of *R. rickettsii* as positive control.

Additionally, amplifications of the mitochondrial 12S rRNA gene with primers T1B/T2A (Burkman et al., 2009) were performed to confirm the taxonomic identification and DNA integrity of random selected samples (data not shown).

Table. Species of potential vector of *Rickettsia* sp. collected in December 2012 at the Pedro Leopoldo Model Farm Sustainable Production Center (Federal University of Minas Gerais), Pedro Leopoldo, Minas Gerais State, Brazil.

	Vectors	(n)				
Environment/ Host (n)	Species	L	N	M	F	Total
Environment (NA)	*Amblyomma sculptum*	272	584	22	6	884
	Dermacentor nitens	0	1	5	1	7
	Rhipicephalus microplus	3	0	0	0	3
	Subtotal	275	585	27	7	894
Bos taurus (4)	*Amblyomma sculptum*	0	0	1	0	1
	Rhipicephalus microplus	0	7	7	18	32
	Subtotal	0	7	8	18	33
Equus caballus (6)	*Amblyomma sculptum*	0	0	9	5	14
	Rhipicephalus microplus	0	0	0	1	1
	Dermacentor nitens	0	0	3	3	6
	Ctenocephalides felis	0	0	1	0	1
	Subtotal	0	0	13	9	22
Ovis aries (3)	*Rhipicephalus microplus*	0	8	0	8	16
	Subtotal	0	8	0	8	16
Canis familiaris (6)	*Amblyomma sculptum*	0	16	17	4	37
	Rhipicephalus microplus	0	0	2	0	2
	Ctenocephalides felis	0	0	6	14	20
	Subtotal	0	16	25	18	59
	Total	275	616	73	60	1024

(n): sample size; NA: not applicable; L: larvae; N: nymph; M: male; F: female.

PCR products were purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega), followed by application of the BigDye™ Terminator–Cycle Sequencing Ready Reaction kit (Applied Biosystems™) and analyzed in an automated ABI 3730xl DNA analyzer (Applied Biosystems™). Sequence edition was performed with SeqMan software (DNASTAR® package, Lasergene), and identity values were obtained by Basic Local Alignment Search Tool (BLAST) analysis (http://blast.ncbi.nlm.nih.gov).

A concatenated sequence was generated with the *gltA* (1104bp) and *htrA* (463bp) genes and the Maximum-Likelihood tree was inferred using PhyML 3.0 online software with GTR+G correction model, selected by Smart Model Selection (Guindon et al., 2010), with bootstrap values obtained from 1,000 randomly generated trees.

RESULTS

One male of *A. sculptum*, collected from a dog, was infected by *Rickettsia*, identified as *Rickettsia* sp. isolate LIC4167A (MH158234 and MH158235).

The sequences of *gltA* and *htrA* genes showed identity values of 100% (1066/1066bp) and 99% (497/498bp), respectively, with the *Rickettsia* endosymbiont of *Amblyomma dubitatum* (Serra dos Órgãos strain) (JN676158) and uncultured *Rickettsia* sp. clone Pampulha (JN190456). Likewise, phylogenetic inferences grouped the isolate LIC4167A with these closely related to *R. tamurae* and *R. monacensis* Brazilian sequences (Figure).

Molecular tests were performed for *OmpA, OmpB* and *Sca4* targets. However, no positive results were obtained for these rickettsial genes, yet the sequences obtained with the other genes were considered sufficiently relevant for the article results.

BLAST analysis of 12S rRNA gene sequences obtained from randomly selected samples (data not shown) (MH790115) revealed high sequence identity values (100%, 340/340bp) with *A. sculptum* sequence (KY172627).

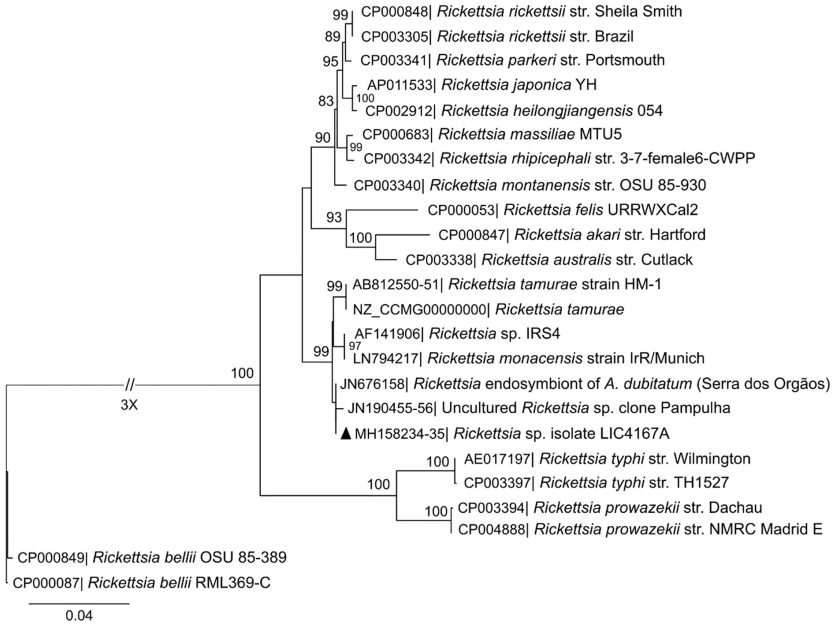

Figure. Phylogenetic tree of concatenated rickettsiae *gltA* and *htrA* genes constructed by Maximum-Likelihood method and GTR+G as evolution model and based on the nucleotide sequences. The GenBank accession codes precede sequence names and the numbers at nodes are bootstrap values obtained from 1,000 re-samplings. Bootstraps values below 70% are not shown. The triangle indicates the isolate detected in the present study. The decrease in the branch corresponds to 3 times the reference bar.

DISCUSSION

The most common BSF epidemiological scenario occurs in anthropic areas of the Cerrado and Atlantic Rainforest biomes, involving the presence of *A. sculptum* and *A. dubitatum* infected with the BSF *R. rickettsi* bioagent mainly parasitizing capybaras and equines (Szabó et al., 2013; Oliveira et al., 2016a). In the Southeast, capybaras are key hosts for these tick species, however, they present significant feeding-behavior differences and, indeed, *A. sculptum* poses a greater risk regarding human biting and thereafter transmitting tick-borne pathogens (Neto et al., 2018).

However, some cases of Spotted Fever in Brazil evidence different forms of disease manifestation, and for these the epidemic or enzootic cycle, as well as the causal agent, remain uncharacterized (Oliveira et al., 2017; Silva et al., 2018). Recent studies have increased awareness of the occurrence of other SFG Rickettsiae circulating in several regions of the country, including those associated or not with BSF human cases (Guedes et al., 2005; Almeida et al., 2011; Szabó et al., 2013; Silva et al., 2018).

The southeastern region of Brazil is the main area for BSF occurrences, and Minas Gerais is one of the Brazilian States with the highest numbers of confirmed cases and deaths (Oliveira et al., 2016a). The majority of these cases have been serologically confirmed, however etiological agent identification is not always performed. In this context, the Belo Horizonte Metropolitan Region has historically been considered endemic for BSF, with recent human cases being reported (Guedes et al., 2005).

SFG *R. tamurae* was initially isolated from *Amblyomma testudinarium* ticks in Japan and human infections were confirmed by molecular and serological analyses (Phongmany et al., 2006, Imaoka et al., 2011). In Brazil, sequences related to *R. tamurae* and *R. monacensis* were obtained from *Amblyomma* spp. collected in Juiz de Fora and from *A. dubitatum* (strain Pampulha), collected in Belo Horizonte, the same region of the sample in this study. Both regions are considered BSF endemic areas of Minas Gerais State (Szabó et al., 2013; Guedes et al., 2011). Furthermore, other strains, related to the *R. tamurae* and *R. monacensis* cluster, were recorded in different eco-epidemiological scenarios, indicating circulation of this species in BSF human case areas. For instance, the Serra dos Órgãos strain was recorded from *Amblyomma* spp. collected in Rio de Janeiro State (Spolidorio et al., 2012), and an *Ixodes aragaoi* was detected with a *Rickettsia* sp. genetically close to *R. monacensis* and *R. tamurae*, named *Rickettsia* sp. strain Aragaoi (Blanco et al., 2016), in Paraná State.

Knowledge of these strains is still incipient and may possibly be the same strain occurring in different vectors and ecosystems. The use of more refined and integrative techniques will allow greater knowledge of the structural complexity of the microbial communities associated with ticks from different ecosystems.

Detection of human-biting ticks infected by this bacterium during BSF surveillance reveals the importance of *A. sculptum* for public health, especially due to its widespread presence in the Brazilian territory and its capacity to transmit Rickettsial organisms. This is the first report of a bacterium phylogenetically related to *R. tamurae* and *R. monacensis* infecting *A. sculptum* in Brazil.

The potential role of this novel Rickettsiae as a human pathogen must be considered, since both *R. tamurae* and *R. monacensis* have been reported infecting humans (Jado et al., 2007; Imaoka et al., 2011). Further studies are needed to identify the cycle of this Rickettsiae and evaluate its importance in endemic areas of BSF cases.

This study looks at the presence of bacterium phylogenetically related to *R. tamurae* and *R. monacensis* infecting *A. sculptum* in Brazil. The potential role of this novel Rickettsiae as a human pathogen must be considered, since both *R. tamurae* and *R. monacensis* have been reported infecting human.

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