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Original article

Spotted fever group *Rickettsia* in *Amblyomma dubitatum* tick from the urban area of Campo Grande, Mato Grosso do Sul, Brazil

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ABSTRACT

Rickettsia infection of each tick was evaluated by the hemolymph test and polymerase chain reaction (PCR) targeting *glt*A and *omp*A genes. All hemolymph tests were negative and PCR of one *A. dubitatum* detected both Rickettsia genes. Sequence of *omp*A exhibited a 99% identity with *Rickettsia parkeri* and *R. africae* and a 98% identity with *R. sibirica*. *Rickettsia* of the spotted fever group in *A. dubitatum* is described for the first time in an urban area within the municipality of Campo Grande in the state of Mato Grosso do Sul (MS), Brazil. This finding reinforces the importance of more detailed studies to determine the role of *A. dubitatum* in the transmission of spotted fever agents.

Introduction

Rickettsioses constitute a group of diseases caused by bacteria of the genus *Rickettsia*, which are Gram-negative, obligate intracellular bacteria that are associated with invertebrate vectors (Raoult and Roux, 1997). Human cases of infection by bacteria of this genus have been described in world widely (Angerami et al., 2012; Kelly et al., 1992; Demma et al., 2006).

In Brazil, the disease caused by the bacteria *Rickettsia rickettsii* is known as Brazilian Spotted Fever (BSF) (Labruna, 2009). The southeast region of Brazil is distinguished by a greater incidence of BSF cases and number of deaths (Del Fiol et al., 2010). The high mortality rate recorded in Brazil is primarily caused by the difficulty of obtaining a rapid and accurate diagnosis when patients affected with this disease present with initial nonspecific symptoms (Spolidorio et al., 2010).

Currently, the main vectors of *R. rickettsii* for humans in Brazil are the tick species *Amblyomma cajennense* and *A. aureolatum* (Ogrzewalska et al., 2012; Guedes et al., 2011). However, *R. rickettsii* and other bacteria from the spotted fever group (SFG) have been found in different species of ticks (Ogrzewalska et al., 2013; Labruna et al., 2011; Almeida et al., 2013a,b).

The capybara (*Hydrochoerus hydrochaeris*) is the primary host of *A. dubitatum* (Nava et al., 2010), and it is also among the main hosts of the adult stage of *A. cajennense* (Pacheco et al., 2007). This rodent is involved in maintaining *R. rickettsii* in nature because of the capybara's ability to act as an amplifying host of *R. rickettsii* (Souza et al., 2009).

Despite the lack of knowledge of the role of *A. dubitatum* in the transmission of BSF, there is speculation regarding its possible involvement in maintaining the enzootic cycle of *R. rickettsii* in nature (Labruna et al., 2004). In this study, the presence of *Rickettsia* species from the SFG in free-living ticks from urban areas is investigated, and we describe the first report of a natural infection of *Rickettia* sp. in *A. dubitatum* in the state of Mato Grosso do Sul.

Materials and methods

Study site

This study was conducted between March 2013 and February 2014 in the municipality of Campo Grande in the state of Mato

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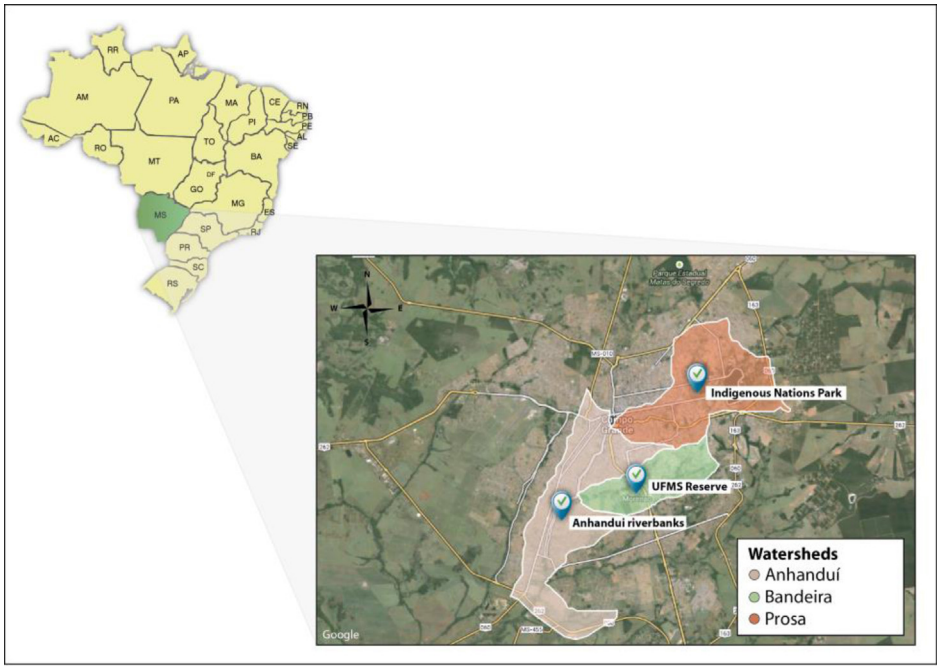

Fig. 1. Collection areas in the municipality of Campo Grande in the state of Mato Grosso do Sul; these areas are influenced by three small watersheds: Anhanduí, Bandeira and Prosa.

Grosso do Sul, Brazil. This municipality is located in the mid-west region of the state, and it contains a population of approximately 832,352 inhabitants (32.1% of the state population) and has an area of 8092.95 km^2. The total urban area is 154.45 km^2, and it is located at an average altitude of 592 m above sea level (IBGE, 2010).

This municipality is included in the Cerrado biome and contains 33 named streams within the urban perimeter that are distributed within ten small watersheds (data from the Municipal Town Hall, available at: http://www.pmcg.ms.gov.br/meioambiente/).

The ticks were collected in three areas [the Indigenous Nations (Nações Indígenas, 20°27′11.2″ S 54°34′00.8″ W) Park, natural reserve of the Federal University of Mato Grosso do Sul (Universidade Federal de Mato Grosso do Sul – UFMS, 20°29′57.4″ S 54°36′54.8″ W) and along the Anhanduí riverbanks (20°30′32.8″ S 54°39′01.9″ W) as part of an ongoing study of the tick fauna of MS. All of the areas are inhabited by capybaras and are considered rest areas for these populations.

Tick collection and identification

Free-living ticks were captured using CO_2 traps as described by Oliveira et al. (2000) taken alive to the tick biology laboratory of Embrapa Beef Cattle and identified according to Barros-Battesti et al. (2006). For the direct detection of *Rickettsia*, each tick was subjected to the hemolymph test (Burgdorfer, 1970) and stained as described by Giménez (1969). Ticks were stored at −80 °C until DNA extraction.

DNA extraction and PCR

The adult ticks were analized individually and the nymphs were analized in group of ten. DNA was extracted from the ticks using the DNAzol Reagent commercial kit Invitrogen (Carlsbard, USA) and subsequently subjected to a polymerase chain reaction (PCR). The oligonucleotides CS-78 and CS-323 were used to amplify a fragment of 401 base pairs (bp) of the citrate synthase gene (*glt*A), which is present in the rickettsial species (Labruna et al., 2004). The PCR was standardized to a final volume of 25 μl, with DNA concentrations between 50 and 250 ng/μl and an A_{260nm}/A_{280nm} ratio ≥1.8.

The positive DNA samples were tested by means of another PCR using the oligonucleotides Rr190.70p and Rr190.602n, which amplify a 530-bp fragment of the *omp*A gene (190-kDa protein) that is only present in the *Rickettsia* species from the SFG (Regnery et al., 1991).

The amplified product of the PCR-*omp*A was purified using the Purelink Kit (Invitrogen) and subsequently cloned and sequenced. Sequencing was performed using the Sanger method (Sanger et al., 1977) in an ABI 3130 Genetic Analyzer (Applied Biosystems). The

Table 1
Tick numbers collected and analyzed by PCR for rickettsial infection in the urban area of Campo Grande, Mato Grosso do Sul state, Brazil, 2013–2014.

Locality	Geographic coordinates	Ticks			
		Species	Tested (*n*)	Infected *n* (%)	*Rickettsia* species
UFMS Reserve	20°29′57.4″ S 54°36′54.8″ W	*Amblyomma cajennense*	785	0	–
		Amblyomma dubitatum	74	0	–
		Amblyomma spp. nymphs	222	0	–
Anhandui riverbanks	20°30′32.8″ S 54°39′01.9″ W	*Amblyomma dubitatum*	10	1 (10)	*Rickettsia* sp.
Indigenous Nations Park	20°27′11.2″ S 54°34′00.8″ W	*Amblyomma dubitatum*	3	0	–
		Amblyomma spp. nymphs	3	0	–
Total			1097	0.09	

Fig. 2. Neighbor-joining phylogram based on partial *omp*A gene sequences showing the phylogenetic position of *Rickettsia* sp. Anhanduí among other *Rickettsia* genotypes. A bootstrap analysis was performed with 1000 repetitions. The number between brackets corresponds to the accession number in GenBank, and the scale bar indicates the nucleotide substitutions per site.

sample was sequenced, and the consensus sequence was obtained using the BioEdit program (Hall, 1999). The obtained sequences were compared to the data from GenBank. A BLASTn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi.) was performed to determine the sequence identity (Altschul et al., 1990), and phylogenetic analyses were conducted using the program MEGA version 3.1 (Kumar et al., 2004).

Results and discussion

A total of 1097 ticks were collected in the three areas during the study period. Only two tick species, *Amblyomma cajennense* and *Amblyomma dubitatum*, were identified, and they were present in the proportion of 9:1, respectively (Table 1).

The species *A. cajennense* recently underwent a taxonomic re-evaluation that resulted in the revalidation of three species and the description and definition of three new species (Nava et al., 2014). The geographic distribution of the six species of the *A. cajennense* complex proposed by Nava et al. (2014) identified the presence of *A. sculptum* and *A. cajennense* s.s. to date in Brazil. *Amblyomma sculptum is* the current specie in this mid-west region, mentioned by the above authors, where this research was realized.

Amblyomma dubitatum is a South American tick species (Guglielmone et al., 2003). In Brazil, the geographic distribution of *A. dubitatum* is concentrated in the provinces of Cerrado, Atlantic Forest, Parana Forest and *Araucaria angustifolia* Forest (Nava et al., 2010). The distribution of *Amblyomma dubitatum* is less restricted than that of its main host, the capybara, which suggests different responses to environmental variables (Nava et al., 2010). Nevertheless, the three studied urban areas that are influenced by three small watersheds (Fig. 1) are inhabited by capybaras and *Amblyomma dubitatum*.

A hemolymph test was performed on the male and female adult ticks, and all of the samples were negative, which is a similar result to that of other authors (Almeida et al., 2013b; Veronez et al., 2010). The difficulty in detecting rickettsias is observed even in endemic areas of spotted fever (Estrada et al., 2006), indicating that a negative result should be analyzed with caution.

PCR of one sample of *A. dubitatum*, collected on the Anhanduí riverbank, detected *Rickettia* genes. Amplification of the *omp*A PCR product placed it in the spotted fever group.

The product generated from the PCR-*omp*A was cloned and sequenced, and after alignment, the generated nucleotide sequences revealed a 99% (489/494) identity with the *omp*A sequences that corresponded to the *R. parkeri* of the Ubatuba (KJ174528) and Necocli (KJ158741) strains found in *A. ovale*, 99% (488/494) identity with *Rickettia S* r*OmpA* (U43805) of *R. sanguineus*, 99% (487/494) identity with *R. africae* (U43790) found in *A. variegatum* and a 98% (484/494) identity with *R. sibirica* (U43807) found in *Dermacentor nuttali* (Fig. 2). The Brazilian SFG isolate identified in this study was designated as *Rickettsia* spp. Anhaduí strain, and the partial sequence of the *omp*A gene was deposited in GenBank with the accession number KJ944582.

The presence of a species of *Rickettsia* from the spotted fever group, designated in this study as the Anhanduí strain, in *A. dubitatum* is reported for the first time in an urban area of the municipality of Campo Grande in the state of Mato Grosso do Sul. The first studies conducted in this state were performed by Ogrzewalska et al. (2013), who detected for the first time the presence of the *Rickettsia parkeri*-like agent that infected *Amblyomma calcaratum* nymphs in birds, and by Almeida et al. (2013a, 2013b), who detected the presence of *Rickettia* spp. in *Amblyomma nodosum* in anteaters and *R. rickettisi* in *R. sanguineus* in dogs of the urban area. All of these results reveal the involvement of different species of ticks in the circulation of SFG agents in different areas of the state of Mato Grosso do Sul.

The possible involvement of *A. dubitatum* as an enzootic vector of the *Rickettsia* species deserves greater attention because a co-infestation of *H. hydrochaeris* with *A. dubitatum* and *A. cajennense* is usually reported (Pacheco et al., 2007; Souza et al., 2009). Moreover, *A. dubitatum* may eventually bite humans (Labruna et al., 2007), and the bite can have induce at different periods of the disease transmission; this observation is based on the work of Guedes and Leite (2008), who noted a differentiated seasonal pattern for the free-living stages of *A. dubitatum* compared to the *A. cajennense* population in an endemic area of spotted fever.

Rickettsia herein described exhibited more than a 98% identity with *R. parkeri*, *R. africae* and *R. sibirica*. Walker and Ismail (2008) and Pacheco et al. (2012) suggested that these three species constitute a single Rickettsia species. These species and closely related Rickettsia are known to cause human rickettsiosis with milder symptoms in several countries including Brazil (Paddock et al., 2004; Parola et al., 2005; Spolidorio et al., 2010, Silva et al., 2011).

Conclusion

In conclusion, *Rickettia* sp. was found to infect *A. dubitatum* under natural conditions in the urban area of Campo Grande, MS, Brazil. This tick species can eventually bite humans, which reinforces the importance of more detailed studies to determine its actual involvement in the transmission of spotted fever agents.

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