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Rickettsia parkeri in *Amblyomma dubitatum* ticks in a spotted fever focus from the Brazilian Pampa

Bárbara Weck[a], Bruno Dall'Agnol[a], Ugo Souza[a], Anelise Webster[a], Bárbara Stenzel[b], Guilherme Klafke[a], João Ricardo Martins[a], José Reck[a,*]

[a] Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Eldorado do Sul, RS, Brazil
[b] Centro Estadual de Vigilância em Saúde (CEVS), Porto Alegre, RS, Brazil

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ABSTRACT

Spotted fever is an acute febrile illness, which is considered severely underreported and misdiagnosed in the Brazilian Pampa, caused by tick-borne *Rickettsiae*. Here, we report an eco-epidemiological investigation of *Rickettsia* spp. in ticks from a spotted fever focus in Toropi, southern Brazil. Ticks were collected from capybara carcasses and processed individually to obtain genomic DNA. *Rickettsia* was investigated using PCR that amplified the rickettsial fragments of the *gltA*, *ompA* and *htrA* genes. DNA from *Rickettsia parkeri* was found in four of 14 *Amblyomma dubitatum* ticks collected from capybara carcasses in Toropi and the nearby municipality of Quevedos. We also tested 210 *A. dubitatum* ticks obtained from road-killed capybaras of other localities from the Pampa biome; none of them were positive for *Rickettsiae*. Thus, in Rio Grande do Sul, two *Rickettsia* species can be potentially associated to spotted fever: *Rickettsia* sp. strain Atlantic Rainforest, associated with *Amblyomma ovale* ticks in the Atlantic Rainforest biome, and *R. parkeri*, associated both with *Amblyomma tigrinum* and *A. dubitatum* ticks in the Pampa biome. Our results reinforce that *R. parkeri* may be the agent associated with spotted fever in the Brazilian Pampa.

1. Introduction

Spotted fever is an acute febrile illness caused by tick-borne *Rickettsiae*. The illness can be fatal if left untreated. In addition, spotted fever is the most important tick-borne disease in South America (Horta, 2004; Labruna, 2009). In Brazil, studies on the ecology of spotted fever vectors have been showed three *Rickettsiae* species associated with disease foci: *Rickettsia rickettsii*, *Rickettsia* sp. strain Atlantic Rainforest and *Rickettsia parkeri* (Labruna, 2009; Spolidorio et al., 2010; Weck et al., 2016)

Rickettsia parkeri was first identified in *Amblyomma maculatum* ticks from Texas, USA, during the 1930s (Parker et al., 1939). Many years later, Paddock and co-workers demonstrated the potential of *R. parkeri* as a human pathogen in the USA after its isolation from a 40-year-old man with eschar-associated febrile illness (Paddock et al., 2004).Since then, several reports have highlighted the high *R. parkeri* infection rate in *A. maculatum* ticks in the USA (Varela-Stokes et al., 2011; Wright et al., 2011). In 2004, *R. parkeri* and *Amblyomma triste* ticks were identified as the major causal agent and vector, respectively, of spotted fever in Uruguay (Venzal et al., 2004). Some years later, *R. parkeri* was also detected in *A. triste* ticks in areas of Argentina, where cases of human spotted fever were reported (Cicuttin and Nava, 2013).

In 2007, Silveira et al. identified *R. parkeri* for the first time in ticks from Brazil. Spolidorio et al. (2010) reported a novel *Rickettsia* species that was genetically closely related to *R. parkeri*; the species was named *Rickettsia* sp. strain Atlantic Rainforest. Currently, strain Atlantic Rainforest has been recognized as one of the major *Rickettsia* species involved in cases of spotted fever in Brazil. Despite this, until 2016 *R. parkeri* sensu stricto (s.s.) was never associated with spotted fever in Brazil. Weck et al. (2016) had proposed that *R. parkeri* s.s. could be associated to spotted fever in the Brazilian Pampa, since it was detected in *A. tigrinum* ticks collected from the domicile of a patient with spotted fever.

Since 2005, spotted fever has been reported in the state of Rio Grande do Sul (RS) in southern Brazil. Even considering its severe underreporting and misdiagnosis, particularly in the Pampa biome, currently approximately 60 suspected human cases have been registered in RS. Unpublished data from the RS Health Department (kindly provided by Centro Estadual de Vigilância em Saúde, CEVS) indicated that there is an increase in the frequency of spotted fever cases and human tick bites in recent years. Here, we reported an eco-epidemiological investigation of *Rickettsia* spp. in ticks from a spotted fever focus

* Corresponding author at: Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Estrada do Conde, 6000, Eldorado do Sul, 92990-000, RS, Brazil.
E-mail address: jose.reck@gmail.com (J. Reck).

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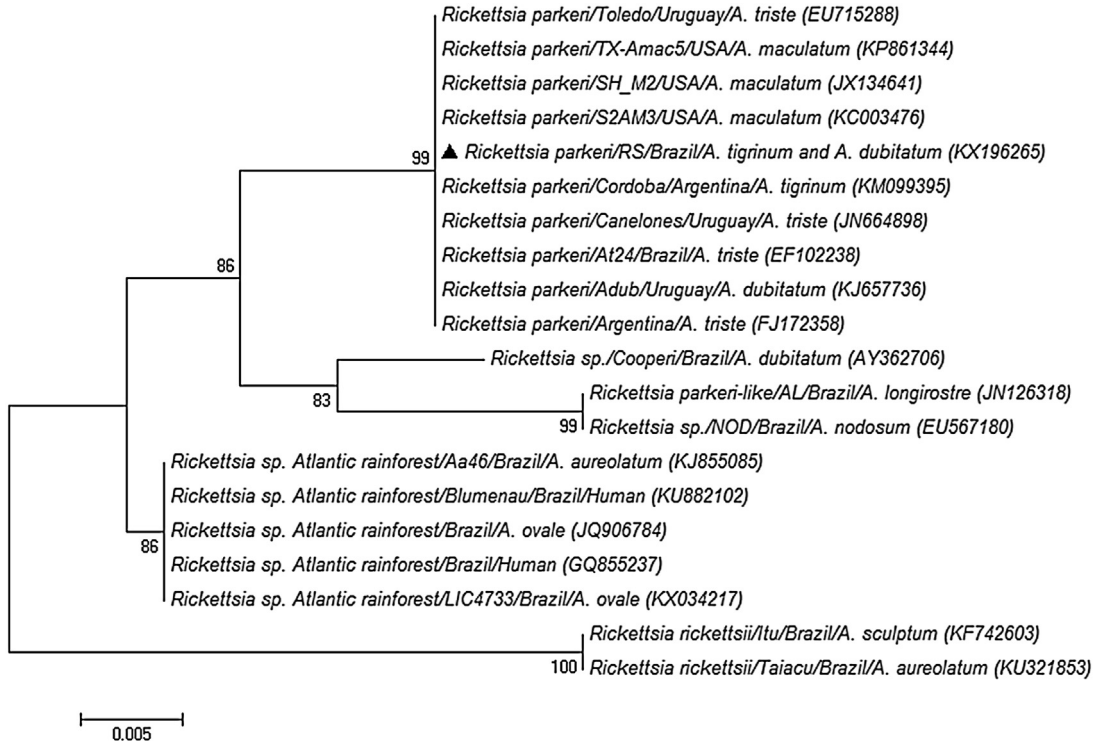

Fig. 1. Phylogenetic tree of the *Rickettsia* spp. *ompA* gene. The analysis was performed using MEGA 7 software using the maximum likelihood algorithm and the Tamura 3-parameter as the DNA substitution model as well as 1000 bootstraps for the phylogeny test. The sample identified in this study is indicated by the black triangle. Sequences were identified by *Rickettsia* species/strain name/country/host. The Genbank accession number is indicated between parentheses.

from the Pampa biome in southern Brazil. The municipality of Toropi was investigated because in 2014, a 4-year-old girl presented fever, conjunctival hyperemia, lymphadenopathy, headache, rash, petechiae, cutaneous eschar in the abdomen and respiratory disturbances. The girl was hospitalized for 2 weeks but after treatment with chloramphenicol showed a complete recovery. The case was confirmed as spotted fever by local health authorities.

Since in Brazil clinical investigation of spotted fever cases does not provide enough information to determine the agent and vector involved; the main part of the current knowledge is based on studies on the ecology of spotted fever vectors. Thus, the aim of this work is to provide information on the vectors and *Rickettsia* species associated with a spotted fever focus in Brazilian Pampa.

2. Materials and methods

2.1. Ticks

Ticks were collected from capybaras in the municipalities of Toropi and Quevedos, during September–December, 2015. Also, capybara ticks from other municipalities of the Pampa biome were obtained from the Tick Collection of the Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF). A total of eight municipalities were sampled: Alegrete (n = 20, 2009), Arroio Grande (n = 11, 2009), Bagé (n = 9, 2016), Cachoeira do Sul (n = 19, 2007–2011), Lavras do Sul (n = 97, 2010–2012), Santa Vitória do Palmar (n = 13, 2009–2011), Santana do Livramento (n = 2, 2014) and Vila Nova do Sul (n = 39, 2015). Ticks were placed in sterile plastic tubes containing absolute ethanol, and stored at −20° C until the tests. Taxonomic identification of ticks was performed according Barros-battesti et al. (2006) and Martins et al. (2010). Whole ticks (adults and nymphs) were processed individually to obtain genomic DNA by a modified version of the Aljanabi and Martinez (1997) salt-extraction technique.

2.2. Molecular detection of Rickettsia spp. and phylogeny

The presence of *Rickettsia* spp. DNA was investigated using a PCR that amplified a fragment (401 bp) of the *gltA* gene. The tick samples that showed a positive amplicon for *Rickettsia* spp. by *gltA* PCR were further tested by a second PCR, which amplified a 617-bp fragment of the *ompA* gene from *Rickettsia* of the spotted fever group. All positive samples were tested again, using primers for a fragment of 549 bp of *htrA*gene to confirm the results and assess any variability between samples. All PCR reactions were conducted as previously described (Supplementary Table 1) (Labruna et al., 2004). The PCR products of the *ompA* and *htrA* genes that matched the expected sizes were purified with the commercial PureLink Quick Gel Extraction & PCR Purification Combo kit (Invitrogen™, Carlsbad, CA, USA), following the manufacturer's recommendations and sent for sequencing at the company ACTGene (Porto Alegre, RS) and then compared with sequences available in Genbank using the BLAST algorithm to determine the species of *Rickettsia*. The phylogenetic tree of the *ompA* gene was built using MEGA 7 software (Kumar et al., 2016).

3. Results

During our first visits to Toropi, no ticks were found in places that the previously mentioned 4-year-old child frequented the most (domicile and school). After an epidemiological investigation, the relatives of the patient (father and grandfather) reported that they regularly hunt capybaras (*Hydrochoerus hydrochaeris*) in Toropi and the nearby municipality of Quevedos. According to them, after a hunt, they always take the intact carcasses to their home for field dressing and skinning procedures. Also, they reported that the carcasses have been frequently parasitized by ticks.

Thus, ticks were obtained from hunted capybaras carcasses. All ticks (five nymphs, two male and seven females) were identified as *Amblyomma dubitatum* ticks. Four samples were positive for the presence of *Rickettsia* spp. DNA – two males from Quevedos and two