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Short communication

First molecular detection of *Rickettsia parkeri* in *Amblyomma tigrinum* and *Amblyomma dubitatum* ticks from Uruguay

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ABSTRACT

Rickettsia parkei is the etiological agent of spotted fever in Uruguay, where is transmitted to humans by the tick *Amblyomma triste*. In the present study, ticks were collected from capybaras (*Hydrochoerus hydrochaeris*) and domestic dogs during 2011–2012 in different parts of Uruguay. Three out of 11 (27.3%) *Amblyomma dubitatum* ticks collected from capybaras, and 4 out of 6 (66.7%) *Amblyomma tigrinum* ticks collected from dogs were shown by molecular analyses to be infected by *Rickettsia parkeri* strain Maculatum 20. Until the present work, *A. triste* was the only tick species that was found infected by *R. parkeri* in Uruguay. This is the first report of *R. parkeri* infecting these two tick species in Uruguay, expanding the current distribution of this rickettsial pathogen in the country.

Introduction

From the early 1990s, cases of tick-borne spotted fever began to be diagnosed in Uruguay, where the disease was initially called as cutaneous ganglionar rickettsiosis (Conti-Díaz et al., 1990). The vector of this disease was later shown to be *Amblyomma triste* (Venzal et al., 2004; Pacheco et al., 2006; Portillo et al., 2013), which is the most frequent human-biting tick in Uruguay (Venzal et al., 2003a,b). The occurrence of tick-borne spotted fever in Uruguay only has been recognized in the coastal counties from the Rio de la Plata to the Atlantic Ocean (Conti-Díaz, 2001; Venzal et al., 2012). Until now, *R. parkeri*-caused rickettsiosis is the only tick-borne disease affecting humans in the country.

This study aimed to report the presence of *R. parkeri* in two other *Amblyomma* species as well as to expand the distribution of *R. parkeri* in Uruguay.

Materials and methods

During studies of ticks that parasitize different animals in Uruguay, ixodids were collected on capybaras (*Hydrochoerus hydrochaeris*) in Sarandí del Yi, Durazno County (33°20′ S; 55°37′ W), on 12 November 2012, on one domestic dog in San Jacinto, Canelones County (34°32′ S; 55°52′ W) on 15 October 2012, and on one domestic dog in Salamanca, Maldonado County (34°12′ S; 54°45′ W) on 19 November 2011. These ticks were preserved in ethanol 96% and sent to the laboratory for analyses. Taxonomic identification was performed under binocular microscope using taxonomic keys of Barros-Battesti et al. (2006) and Martins et al. (2014). Blood from San Jacinto's dog was collected and sent to the laboratory for serological analyses.

In order to be processed by molecular techniques, tick DNA was extracted using a commercial kit (Promega®), according to manufacturer's instructions. Subsequently, conventional PCR was performed to detect the presence of rickettsial DNA in the ticks. Firstly, a PCR targeting a fragment of rickettsial *gltA* gene, which codes for the citrate synthase enzyme, was performed. The primers used were CS-78 and CS-323 (Labruna et al., 2004) which amplifies a fragment of 401 bp. The PCR-positive samples were subjected to a second PCR targeting a fragment of the rickettsial 190-kDa gene (*ompA*). In this case, the Rr 190.70 and Rr 190.602 primers were employed (Regnery et al., 1991), amplifying a 532 bp fragment, present only in *Rickettsia* species belonging to spotted fever group (SFG). PCR amplicons were purified and sequenced as previously reported (Labruna et al., 2004). Generated sequences were submitted for basic local alignment search tool (BLAST) analysis

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(Altschul et al., 1990) to determine closest similarities to available corresponding DNA sequences in GenBank.

Serum was processed using Indirect Immunofluorescence Assay (IFA), following the protocol of previous descriptions (Zavala-Velazquez et al., 1996; Horta et al., 2004; Labruna et al., 2007). In this investigation antigens corresponding to three *Rickettsia* species were used: *R. rhipicephali, R. felis* and *R. parkeri*, from HJ5, Pedreira and AT24 strains respectively. Canine serum endpoint titer against each *Rickettsia* strain was determined by testing serial twofold serum dilutions starting from a 1:64 dilution. To suggest the species involved on the immunological response it was considered that the serum had to show a titer for a *Rickettsia* species at least four-fold that observed for any other *Rickettsia* species, as previously reported (Horta et al., 2004, 2007; Labruna et al., 2007; Pinter et al., 2008; Pena et al., 2009).

Results and discussion

All ticks collected from the animals were adults of the genus *Amblyomma*, as follows: five males and six females (none of them engorged) of *Amblyomma dubitatum* from capybaras in Durazno County; two males and three females (two of them partially engorged) of *Amblyomma tigrinum* from one dog in Canelones County; and one female (not engorged) of *A. tigrinum* and one female of *A. aureolatum* (engorged) from a dog in Maldonado County. No *A. triste* ticks were collected in any of the hosts mentioned.

Three (two non engorged females and one male) out of 11 (27.3%) *A. dubitatum* ticks, and four (one partially engorged female and two males from San Jacinto's dog; and, one non engorged female from Salamanca's host) out of six (66.7%) *A. tigrinum* ticks yielded amplicons for both *gltA* and *ompA* PCR assays. Nucleotide sequence analysis by BLAST revealed that the *Rickettsia* species infecting all PCR positive ticks was *R. parkeri* strain Maculatum 20, with 100% similarity for both genes (GenBank accession numbers U59732 and U43802). GenBank nucleotide sequence accession numbers for the partial sequences generated in this study are KJ657733 and KJ657734 for partial sequences of *R. parkeri gltA* and *ompA* genes, respectively, from *A. tigrinum*, and KJ657735 and KJ657736 for partial sequences of *R. parkeri gltA* and *ompA* genes, respectively, from *A. dubitatum*.

The serum analysis of the dog from San Jacinto suggests that this host had previously been in contact with *R. parkeri*. Through IFA, a titer of 4096 for that pathogen was determined, being higher than four-fold when compared to the other species tested (256 for *R. rhipicephali* and 128 for *R. felis*), thus strongly indicating *R. parkeri* as the responsible for the immune response evidenced.

Until the present work, *A. triste* was the only tick species that was found infected by *R. parkeri* in Uruguay (Venzal et al., 2012).

The present study adds two additional tick species, *A. dubitatum* and *A. tigrinum*. It worth mentioning that as all the ixodids were collected on hosts, the possibility of acquiring the infection by blood meal exists. Nevertheless, most of the ticks were not engorged (when females) at the time of collection. Molecular detection of *R. parkeri* Maculatum 20 strain-like in *A. tigrinum* was previously reported in Bolivia (Tomassone et al., 2010); however, such rickettsial strain was never reported in *A. dubitatum* ticks. Interestingly, a *R. parkeri*-close related agent (strain Cooperi) was found infecting *A. dubitatum* ticks in Brazil (Labruna et al., 2004). While the *gltA* partial sequence of *R. parkeri* generated from *A. dubitatum* in the present study is 100% identical to both strain Maculatum 20 (U59732) and strain Cooperi (AY362704), the *ompA* partial sequence is 100% identical to strain Maculatum 20 (U43802) and 98% to strain Cooperi (AY362706). Indeed, this polymorphism in the *ompA* partial sequences supports our first report of a *R. parkeri* Maculatum 20 strain-like in *A. dubitatum* ticks.

The *R. parkeri*-infected *A. tigrinum* ticks were collected in sites within the distribution area of *A. triste* in Uruguay (Venzal et al., 2012), suggesting that *R. parkeri* could be circulating between these two tick species in the same areas. These fact is supported by the fact that *A. triste* and *A. tigrinum* share several host species for both immature and adult ticks in these areas (Venzal et al., 2003a; Martins et al., 2014). On the other hand, our findings of *R. parkeri*-infected *A. dubitatum* ticks came from areas outside the distribution of *A. triste* and spotted fever (Conti-Díaz, 2001; Venzal et al., 2003a; Martins et al., 2014), expanding the distribution of *R. parkeri* in Uruguay.

Because both *A. tigrinum* and *A. dubitatum* are human-biting ticks (Venzal et al., 2003a,b; Labruna et al., 2007), albeit much less frequently than *A. triste*, the results of this study could have epidemiological importance for the natural history of tick-borne spotted fever in Uruguay. Similarly, the distribution of those two tick species is different and wider compared to *A. triste*'s geographical range. While the last is confined only to the coastal counties (Canelones, Colonia, Maldonado, Montevideo, San José and Rocha), *A. dubitatum* is present in Canelones, Durazno, Flores, Lavalleja, Rocha, Tacuarembó and Treinta Tres y, and *A. tigrinum*'s distribution comprises almost all Uruguayan counties (Artigas, Canelones, Cerro Largo, Colonia, Durazno, Flores, Florida, Lavalleja, Maldonado, Montevideo, Paysandú, Rocha, Salto, San José and Soriano) (Venzal et al., 2003a; Martins et al., 2014).

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