Molecular identification of *Rickettsia parkeri* infecting *Amblyomma triste* ticks in an area of Argentina where cases of rickettsiosis were diagnosed

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Specimens of the hard tick Amblyomma triste *were found infected with* Rickettsia parkeri *in an area of Argentina (General Lavalle, Buenos Aires Province) where cases of human illness attributed to this microorganism have been reported. Molecular detection of* R. parkeri *was based on polymerase chain reactions that amplify a ca. 400-bp fragment of the 23S-5S intergenic spacer and a ca. 500-bp fragment of the gene encoding a 190-kDa outer membrane protein. Three (6.97%) of 43* A. triste *ticks were determined to be positive for* R. parkeri. *These results provide strong evidence that* A. triste *is the vector of* R. parkeri *in the study area. The findings of this work have epidemiological relevance because human parasitism by* A. triste *ticks has been frequently recorded in some riparian areas of Argentina and Uruguay and new cases of* R. parkeri *rickettsiosis might arise in the South American localities where humans are exposed to the bites of this tick species.*

Key words: *Rickettsia parkeri* - *Amblyomma triste* - Argentina

Bacteria of the genus *Rickettsia* (Rickettsiales: Rickettsiaceae) are obligate intracellular parasites of eukaryotic cells. Currently, *Rickettsia* species are classified into a variety of groups, including the spotted fever group (SFG), typhus group, transitional group (TRG) and ancestral groups (Gillespie et al. 2007, Weinert et al. 2009). In Latin America, three species of SFG rickettsiae (*Rickettsia rickettsii*, *Rickettsia parkeri* and *Rickettsia massiliae*) were recognised as agents of human disease and hard ticks of the genera *Amblyomma* and *Rhipicephalus* are the vectors of these pathogenic microorganisms (Labruna et al. 2011). In Argentina, human rickettsiosis caused by *R. rickettsii* was diagnosed in Jujuy Province (Paddock et al. 2008) and cases of human illness attributed to *R. parkeri* have been reported in the provinces of Buenos Aires, Entre Rios and Chaco (Romer et al. 2011). The vectors involved in the transmission of *R. rickettsii* and *R. parkeri* in Argentina are the ticks *Amblyomma cajennense* and *Amblyomma triste*, respectively (Nava et al. 2008, Paddock et al. 2008, Labruna et al. 2011, Romer et al. 2011). Additionally, a recent report documented human infection with *R. massiliae* in Argentina (García-García et al. 2010), although new data are needed to confirm the epidemiological relevance of this case.

Although the cases of *R. parkeri* infection in humans were diagnosed in different localities within Argentina (Romer et al. 2011), no data exist regarding the potential vectors in these areas. The only exception is the finding of Nava et al. (2008) in the Paraná Delta region of Buenos Aires Province, where *A. triste* ticks were found infected with *R. parkeri*. The other reports of *R. parkeri* rickettsiosis in Buenos Aires Province correspond to sites located approximately 350 km to the south of the Paraná Delta in the Bahia Samborombón Region (General Lavalle and Verónica); however, descriptions of clinical cases constitute the only available information (Romer et al. 2011). Therefore, a survey of ticks was conducted during August and September of 2011 in General Lavalle (36º22'S 56º21'W), Buenos Aires Province. The study area is located on the western coast of the Rio de la Plata estuary and is characterised by the presence of intertidal mudflats and creeks, freshwater lagoons and marshes and slow-flowing streams. The vegetation in this area is dominated by grasslands, small groups of trees (principally *Celtis tala*) and shrubs. Human activities related to recreation and livestock production are common at this site.

Questing adult ticks were collected from vegetation by dragging and were subsequently preserved in 96% ethanol. A total of 51 specimens were collected and determined to be adults of *A. triste* based on the methods of Estrada-Peña et al. (2005); of these 51 specimens, 43 were individually tested for *Rickettsia* spp infection by polymerase chain reaction (PCR). DNA was extracted using the AxyPrep Multisource Genomic DNA Miniprep kit (Axygen Biosciences, USA) according to the manufacturer's instructions and eluted in a final volume of 100 µL. Detection of *Rickettsia* spp was based on a PCR that amplifies a ca. 400-bp fragment of the 23S-5S intergenic spacer using the primers RCK/23-5-F: GATAGGTCRGRTGTGGAAGCAC and RCK/23-5-R: TCGGGAYGGGATCGTGTGTTTC (Jado et al. 2006). PCR-positive samples were used to amplify a ca. 500-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) with the

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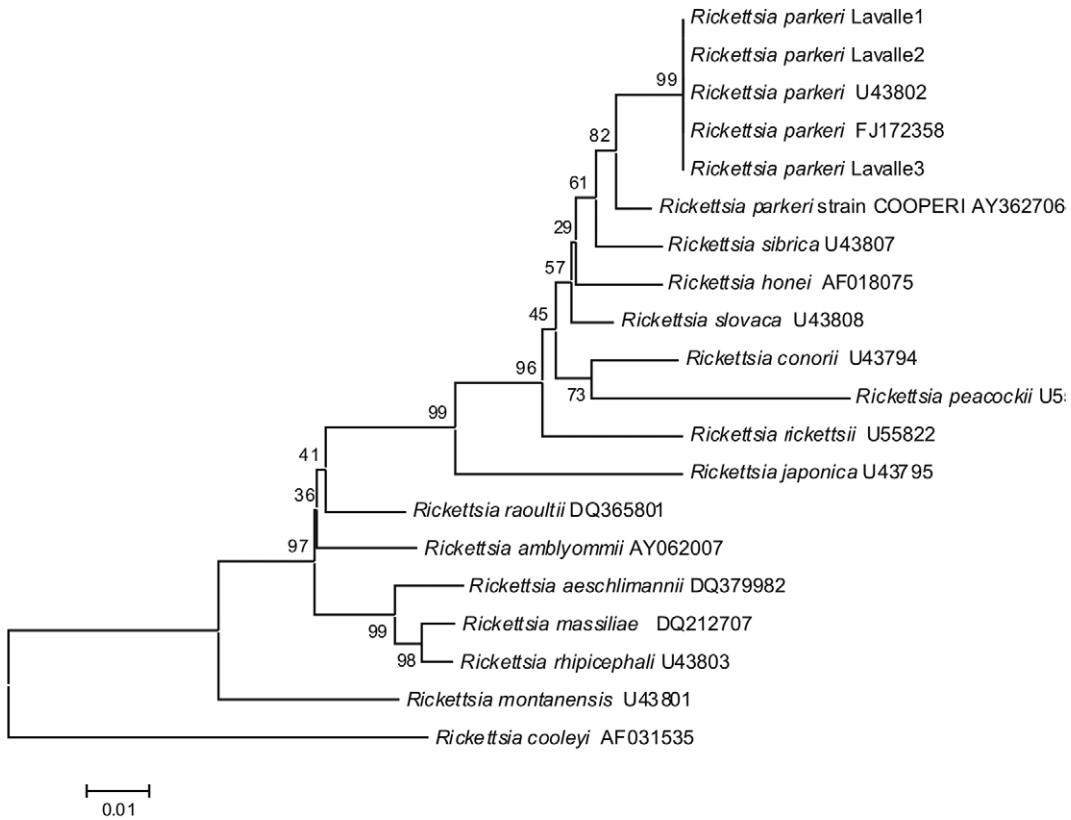
primers Rr190.70p: ATGGCGAATATTTCTCCAAAA and Rr190.602n: AGTGCAGCATTCGCTCCCCCT (Regnery et al. 1991). All amplicons were purified and sequenced. Sequencing reactions were performed using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit and an Applied Biosystems 373A gene sequencer. The sequences were aligned with each other and with the corresponding sequences of the *Rickettsia* species available in GenBank. A phylogenetic analysis was performed using the neighbour-joining (NJ) method with the program Mega 4.0 (Tamura et al. 2007). The NJ tree was generated using the Tamura-Nei model, gaps were excluded in the pairwise comparison and support for the topology was tested using 1,000 bootstrap replications.

Amplicons were obtained from the DNA extracts of three (6.97%) of the 43 *A. triste* ticks. The samples that were positive for the 23S-5S intergenic spacer were used to obtain sequences of the *ompA* gene fragment (sequences submitted to GenBank: JX534933, JX534932). The three sequences were identical with each other and with the *ompA* sequences of *R. parkeri* from the United States (U43802) and from Paraná Delta in Buenos Aires Province (FJ172358). The NJ tree obtained with partial *ompA* sequences of *Rickettsia* species is illustrated in the Figure.

This work provides strong evidence that *A. triste* is the vector of *R. parkeri* in the study area. These results are not unexpected because *A. triste* is known to be the principal vector of *R. parkeri* in Brazil (Silveira et al. 2007, Labruna et al. 2011), Uruguay (Venzal et al. 2004, Pacheco et al. 2006) and other areas of Argentina in localities belonging to the lower Paraná River Delta region in northeastern Buenos Aires Province (Nava et al. 2008, Romer et al. 2011). The prevalence of *A. triste* ticks infected with *R. parkeri* in General Lavalle was similar to that recorded in previous studies performed in Argentina (Nava et al. 2008), Brazil (Silveira et al. 2007) and Uruguay (Pacheco et la. 2006), where the values ranged from 2.6-9.7%.

A. triste has a one-year life cycle in the Southern Cone of South America; immature stages are principally active in the summer, whereas adults, which frequently bite humans, are more abundant from late winter to midspring (Venzal et al. 2008, Nava et al. 2011). Therefore, the risk of human infestation by *A. triste* ticks increases during the part of the year when adults reach their peak abundance. Thus, most of the cases of disease in humans that are attributed to infection with *R. parkeri* are reported in spring and early summer (Venzal et al. 2004, Seijo et al. 2007, Conti-Díaz et al. 2009).

Information from previous studies in Argentina, Brazil and Uruguay (Venzal et al. 2004, Pacheco et al. 2006, Silveira et al. 2007, Nava et al. 2008, Widmer et al. 2011) along with the results obtained in this study suggest that the infection of *A. triste* ticks with *R. parkeri* is a ubiquitous phenomenon. Particularly in Argentina, *A. triste* is present in wetlands and environments that are prone to flooding, as demonstrated by records in the administra-



Neighbour-joining phylogenetic tree obtained from partial outer membrane protein gene sequences showing the phylogenetic position of *Rickettsia parkeri* from General Lavalle (Buenos Aires Province, Argentina) among known *Rickettsia* species.

tive provinces of Buenos Aires, Corrientes, Entre Rios and Formosa (Nava et al. 2011). Because human parasitism by *A. triste* adults is frequent in some riparian areas where this tick is present (Nava et al. 2011), new cases of *R. parkeri* rickettsiosis might arise in the localities where humans are exposed to the bites of *A. triste* ticks.

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