**Rickettsia parkeri** Rickettsiosis in Different Ecological Regions of Argentina and Its Association with *Amblyomma tristis* as a Potential Vector

Yamila Romer,* Santiago Nava, Francisco Govedic, Gabriel Cicuttin, Amy M. Denison, Joseph Singleton, Aubree J. Kelly, Cecilia Y. Kato, and Christopher D. Paddock

Hospital F. J. Muñiz, Buenos Aires, Argentina; Instituto Nacional de Tecnología Agropecuaria, Consejo Nacional de Investigaciones Científicas y Técnicas, Rafaela, Santa Fe, Argentina; Sanatorio Allende, Córdoba, Argentina; Instituto de Zoonosis Luis Pasteur, Buenos Aires, Argentina; Infectious Diseases Pathology Branch and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia

**Abstract.** *Rickettsia parkeri*, a newly recognized tick-borne pathogen of humans in the Americas, is a confirmed cause of spotted fever group rickettsiosis in Argentina. Until recently, almost all cases of *R. parkeri* rickettsiosis in Argentina have originated from the Paraná River Delta, where entomological surveys have identified populations of *R. parkeri*-infected *Amblyomma triste* ticks. In this report, we describe confirmed cases of *R. parkeri* rickettsiosis from Córdoba and La Rioja provinces, which are located several hundred kilometers inland, and in a more arid ecological region, where *A. triste* ticks do not occur. Additionally, we identified questing *A. tigrinum* ticks naturally infected with *R. parkeri* in Córdoba province. These data provide evidence that another human-biting tick species serves as a potential vector of *R. parkeri* in Argentina and possibly, other countries of South America.

**INTRODUCTION**

*Rickettsia parkeri*, a newly recognized spotted fever group (SFG) rickettsial pathogen of humans in the Americas, was first confirmed as a cause of disease in Argentina in 2011, when several cases were identified in patients who were bitten by ticks in the Paraná River Delta.¹ This large wetland expanse, located approximately 130 km northwest of the city of Buenos Aires, is characterized by a temperate climate with abundant annual rainfall and contains an intricate network of tidal channels, marshes, and islands.² Almost all cases of *R. parkeri* rickettsiosis in Argentina have originated from the Paraná River Delta,¹⁰ and entomological surveys have identified populations of *R. parkeri*-infected *Amblyomma triste* ticks from this region;³ nonetheless, a few suspected cases have been recognized recently in areas far beyond the boundaries of Paraná River Delta, including the Bahía Samborombón area in the province of Buenos Aires and the Traslasierra Valley in the province of Córdoba.¹¹

Herein, we present four confirmed cases of *R. parkeri* rickettsiosis, including three cases acquired in an ecological region considerably different from the recognized habitat of *A. triste*⁵ and located several hundred kilometers inland from where *R. parkeri* rickettsiosis is considered endemic in Argentina. Additionally, we identify another human-biting tick species in this region that represents a second potential vector of *R. parkeri* in Argentina and possibly, other countries of South America.

**MATERIALS AND METHODS**

Serological and molecular analysis of patient specimens.

Four patients were referred to the Zoonosis Service at the F. J. Muñiz Hospital, Buenos Aires, Argentina, during 2011–2013 for evaluation of illnesses characterized by fever, rash, and one or more inoculation eschars that followed a tick bite. Patients were bitten by ticks in widely separated geographical regions in the provinces of Buenos Aires, Córdoba, and La Rioja. From each patient, serum and skin biopsy specimens were obtained for serological and molecular analysis. Biopsy specimens of inoculation eschar lesions were obtained by using a 6-mm skin punch. DNA was extracted from these specimens by using a QIAamp DNA Mini Kit, (QIAGEN, Valencia, CA) and 2.5 µL of each extract was tested by using a real-time polymerase chain reaction (PCR) assay targeting a 96-bp segment of the *R. parkeri ompB* gene and a 111-bp segment of a hypothetical gene of *R. rickettsii*.⁷ A C₅ₐ (threshold cycle) value < 40 was considered positive. Serum samples were collected during the acute and convalescent phases of the patients’ illnesses and tested for immunoglobulin G (IgG) antibodies reactive with antigens of *R. parkeri* and *R. rickettsii* by using an indirect immunofluorescence antibody (IFA) assay⁹ starting at an initial dilution of 1:32. Antibody titers were expressed as the reciprocal of the last dilution exhibiting specific fluorescence. An antibody titer ≥ 64 to one or both antigens was considered evidence of exposure to an SFG *Rickettsia* species. A fourfold or greater change in titer between specimens collected separately was considered evidence of a recent infection with an SFG *Rickettsia* species.

**Tick collection and molecular analysis.** During September of 2011, 20 free-living, adult-stage *A. triste* ticks were collected from vegetation in Punta Indio by using cloth drags. Specimens were stored at −20°C and later processed as two pools of 10 ticks each. DNA was extracted using a NucleoSpin Tissue DNA Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) according to the manufacturer’s instructions and eluted in a final volume of 100 µL. During October of 2013, 102 free-living, adult-stage *A. tigrinum* ticks were collected by dragging vegetation in the Traslasierra Valley. Specimens were preserved in 96% ethanol and identified to species using accepted morphological features.⁸ DNA was extracted individually from 81 specimens using the Axyprep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences Inc., Union City, CA), and each sample was eluted in a final volume of 100 µL. DNA specimens were screened for *Rickettsia* species using a PCR assay with primers RCK/23S-5-F and RCK/23S-5-R that amplify a segment of the 23S-5S intergenic spacer.¹⁰ Positive samples were subsequently tested by a PCR assay with primers Rr190.70F and Rr190.60Rn to amplify a segment of the 190-kDa outer-membrane protein

*Address correspondence to Yamila Romer, Hospital F. J. Muñiz, Uspallata 2272, 1282 Buenos Aires, Argentina. E-mail: yromer@hotmail.com*
Selected epidemiological, clinical, and laboratory features of four Argentina patients with *R. parkeri* rickettsiosis

<table>
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<th>Feature</th>
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<td>La Rioja</td>
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<td>1,024</td>
<td>128, 512</td>
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<td><em>R. rickettsii</em></td>
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<tr>
<td><em>R. parkeri</em></td>
<td>&lt; 32, 512</td>
<td>256</td>
<td>1,024</td>
<td>256, 512</td>
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</tbody>
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*Acute- and convalescent-phase IgG antibody titers are represented as the reciprocal of the last serum dilution exhibiting a specific pattern of fluorescence when reacted with the antigen.

RESULTS

Patient 1 was a 47-year-old man from Punta Indio in Buenos Aires province (35°16’ S, 57°14’ W) who presented in November of 2011 with fevers as high as 40°C, headaches, myalgia, and a pruritic rash (Table 1). Five days before the onset of fever, he had removed a tick attached near his ear. Physical examination revealed three painless inoculation eschars on the left arm, abdomen, and back that measured 1.0–1.5 cm (Figure 1A) and a sparse vesicular rash involving predominantly the trunk and upper extremities. There was no lymphadenopathy. His serum chemistries and blood cell counts were within normal range. Doxycycline was administered, and he became afebrile within 48 hours. No antibodies reactive with *R. rickettsii* or *R. parkeri* antigens were detected in the serum sample obtained on the 5th day of illness; however, the convalescent serum specimen, collected 15 days after illness onset, revealed antibody titers to *R. rickettsii* and *R. parkeri* antigens of 128 and 512, respectively. Real-time PCR analysis of DNA extracted from an inoculation eschar biopsy specimen was positive for *R. parkeri* and negative for *R. rickettsii*.

Patient 2 was a 57-year-old woman from the town of Nono in Córdoba province (31°59’ S, 64°22’ W) who presented in December of 2012 with 7 days of fever and generalized rash. Three days before the onset of symptoms, she noted a tick attached to her abdomen while working in her yard. She was initially evaluated at a community clinic, where she was referred for additional evaluation. Physical examination revealed a temperature of 39°C, a 1.5-cm painless eschar on her abdomen (Figure 1B), and a sparse maculopapular and vesicular rash involving her trunk and limbs. There was no lymphadenopathy. Except for a prolonged erythrocyte sedimentation rate of 50 mm/hour (normal = 1–20 mm/hour), her serum chemistries and blood cell counts were normal. Empirical treatment with doxycycline was initiated, and her fever resolved within 48 hours. A serum specimen obtained on day 8 of her illness revealed antibodies reactive with *R. rickettsii* and *R. parkeri* antigens at titers of 32 and 256, respectively. PCR evaluation of the inoculation eschar biopsy specimen was positive for infection with *R. parkeri* and negative for *R. rickettsii*.

Patient 3 was a 47-year-old man from Valle de Calamuchita (31°59’ S, 64°22’ W) in Córdoba province who presented in November of 2013 after 10 days of fever and rash. Five days before the start of fever, he removed a tick from behind his right knee. Physical examination revealed a temperature of 38°C, a tender 1-cm eschar in his popliteal fossa, and a sparse maculopapular and vesicular rash involving his trunk and limbs that spared his palms and soles. There was no lymphadenopathy. His serum chemistries and blood cell counts were normal. He was started on doxycycline, and his symptoms resolved in 48 hours. A biopsy specimen of the eschar was obtained on day 10 of fever, and a serum sample was collected 30 days later. His serum contained antibodies reactive with *R. rickettsii* and *R. parkeri* at titers of 1,024 to each antigen. Real-time PCR evaluation of the eschar biopsy specimen was positive for infection with *R. parkeri* and negative for *R. rickettsii*.

Patient 4 was a 28-year-old man who was bitten by a tick in November of 2013 while camping in Malanzán (30°48’ S, 66°36’ W) in La Rioja province. Approximately 1 week later, he developed fever, myalgia, and a lesion on his neck. A non-pruritic

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*Figure 1. Appearance of inoculation eschars of *R. parkeri* rickettsiosis. (A) An eschar on the abdomen of patient 1 from the Bahía Samborombón region of Buenos Aires province. (B) An eschar on the abdomen of patient 2 from the Traslasierra Valley of Córdoba province.*
rash developed on his body approximately 5 days later. Physical examination revealed a temperature of 40°C, a small tender eschar on his posterior cervical region, and a maculopapular exanthem involving his trunk, limbs, and face that spared his palms and soles. There was no lymphadenopathy. His serum chemistries and blood cell counts were normal. Empirical treatment with doxycycline was started, and his symptoms resolved in 72 hours. A biopsy specimen of the eschar and a serum sample were obtained on day 10 of fever, and a convalescent-phase serum sample was collected 30 days later. His acute- and convalescent-phase serum samples reacted with R. parkeri in 72 hours. A biopsy specimen of the eschar and a serum sample were obtained on day 10 of fever, and a convalescent-phase serum sample was collected 30 days later. His acute- and convalescent-phase serum samples reacted with R. parkeri at titers of 128 and 512, respectively, and R. parkeri convalescent-phase serum samples reacted with R. parkeri and negative for R. rickettsii.

DNA of R. parkeri was detected by PCR in one pool of A. triste ticks from Punta Indio, representing a minimum infection rate of 5%. DNA of R. parkeri was amplified from 2 (2.5%) of 81 questing A. tigrinum ticks collected from Traslasierra Valley. Both ompA sequences were identical with each other (GenBank accession number KM099395) and those ompA sequences of R. parkeri from A. triste ticks from Buenos Aires province4,15 and A. maculatum ticks from the United States.16

DISCUSSION

Since 1999, three pathogenic SFG Rickettsia species, namely R. rickettsii, R. massiliae, and R. parkeri, have been identified as causes of tick-borne illnesses of humans in Argentina.1,3,5,17-19 Rickettsioses are diagnosed infrequently in most South American countries, despite growing recognition that the tick fauna of this continent contains a diverse group of rickettsial pathogens.20 A contributing factor is the coexistence and sympatric distribution of other clinically similar and highly prevalent infectious diseases, such as dengue and leptospirosis.1,18 Clinicians are better positioned to correctly diagnose rickettsioses in locations where pathogenic Rickettsia species have been identified previously in human and arthropod hosts. This study and others provide evidence that R. parkeri is endemic to and infects humans in at least four provinces of Argentina.

The clinical characteristics shared by these patients included moderate fever, one or more inoculation eschars, and maculopapular or vesicular rash. These features are consistent with those described previously in larger series of R. parkeri-infected patients from the Paraná River Delta1,3 and the United States.8,21,22 Regional lymphadenopathy was not identified in any of the patients described in this series, and it had been documented in fewer than 30% of cases reported from Argentina and the United States. For reasons currently unknown, regional lymphadenopathy is reported with much greater frequency among R. parkeri-infected patients in Uruguay.23,24

Molecular confirmation of R. parkeri infection in a patient and a pool of A. triste ticks from Punta Indio (approximately 160 km southeast of the city of Buenos Aires) corroborates a previous investigation that identified patients with an eschar-associated SFG rickettsiosis from Verónica and General Lavalle in Buenos Aires province.1 All of these locations share environmental features with the Paraná River Delta, including grasslands, freshwater lagoons, intertidal mudflats, and marshes, and are included within the Delta e Islas del Paraná ecoregion.23 In addition, recent entomological investigations have identified populations of R. parkeri-infected A. triste ticks from these same areas.4,15 In Argentina, A. triste is found predominantly in wetland environments in the provinces of Buenos Aires, Corrientes, Entre Ríos, and Formosa, where the immature stages parasitize a broad range of sigmodontine and caviid rodents and the adult stages parasitize many domesticated and wild large mammals.5 A. triste is an efficient host and vector of R. parkeri,26 and natural infections of this tick also occur in areas of Uruguay and Brazil, where R. parkeri rickettsiosis is endemic.7-31 Córdoba province, located in the central aspect of Argentina, is the second most populous province in the country and includes many scenic areas that are visited frequently by tourists. The Traslasierra Valley is one of these regions, and it is situated between the Sierra de Córdoba and Sierra de San Luis mountain ranges (approximately 600 km inland from the Paraná River Delta). Malanzañ is located in the southern aspect of La Rioja province (approximately 850 km from the Paraná River Delta). This semi-arid region is also visited by many tourists. These areas comprise several striking contrasts from the alluvial wetland ecosystems of the coastal provinces from where all previously confirmed cases of R. parkeri rickettsiosis in Argentina have originated. The Traslasierra Valley and Malanzañ are situated in the Chaco Seco ecoregion,25 and populations of A. triste do not occur in this part of Argentina; however, A. tigrinum is collected commonly throughout Córdoba province26 and from various sites in La Rioja province (Nava S, unpublished data).

R. parkeri has been detected previously in A. tigrinum ticks attached to dogs in Bolivia and Uruguay,33,34 but this is the first report to describe R. parkeri infection in questing A. tigrinum ticks and document natural infections in specimens of A. tigrinum collected from an area where confirmed human cases of R. parkeri rickettsiosis were also identified. Additional surveys are needed to more fully assess the infection prevalence of R. parkeri in A. tigrinum; nonetheless, the prevalence in specimens collected from the Traslasierra Valley was slightly lower than the percentages of R. parkeri-infected A. triste reported from Argentina (approximately 7–8%).4,15 and other countries in South America (approximately 3–10%).27,28,31 and much lower than the percentage of infected A. maculatum ticks (approximately 10–56%) in the United States.16,35 The phylogenetic relationships among these three vector species are very close, and the morphological differences are subtle; for many years, specimens of A. tigrinum and A. triste (Figure 2) were misidentified frequently as A. maculatum.36

A. tigrinum shows a remarkable ability to adapt to different ecosystems with contrasting climatic conditions.37 In Argentina, small rodents and birds are the principal hosts for larvae and nymphs of A. tigrinum. Adults are commonly found on wild and domestic carnivores and occasionally, other large mammals, including humans.38-41 A. tigrinum occurs in all of the phytogeographical domains of Argentina north of 40°S,7,41 which suggests that the geographical distribution of R. parkeri rickettsiosis in Argentina is more extensive than previously recognized. Additionally, there are records of attachment of A. tigrinum to humans in Brazil, Chile, French Guiana, Paraguay, and Uruguay,40 which suggest that its role in the transmission of R. parkeri to humans may extend to other countries in South America. For example, R. parkeri
rickettsiosis is well-recognized in Uruguay, where parasitism of humans by coastal populations of \textit{A. triste} and inland populations of \textit{A. trigrinum} occur in a manner similar to Argentina. The recent discoveries of diverse tick-borne agents in Argentine ticks that now include various \textit{Rickettsia}, \textit{Coxiella}, and \textit{Ehrlichia} species reflect the increasing awareness of tick-associated diseases throughout Latin America. In this context, enhanced efforts are needed to accurately diagnose and better characterize the scope and magnitude of these complex zoonoses.

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Authors’ addresses: Yamila Romer, Infectious Diseases, Hospital F. J. Muñiz, Buenos Aires, Argentina, E-mail: yromer@hotmail.com. Santiago Nava, Instituto Nacional de Tecnología Agropecuaria, Laboratorio de Parasitología e Inmunología Rafaela, Santa Fe, Argentina, E-mail: nava.santiago@inta.gob.ar. Francisco Govedic, Infectious Diseases, Sanatorio Allende, Córdoba, Argentina, E-mail: frangovedic@hotmail.com. Gabriel Cicuttin, Zoonoses, Instituto de Zoonosis Luis Pasteur, Buenos Aires, Argentina, E-mail: gcicuttin@gmail.com. Amy M. Denison, Infectious Diseases Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: crk6@cdc.gov. Joseph Singleton, Cecilia Y. Kato, and Christopher D. Paddock, Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: jsingleton@cdc.gov, hex0@cdc.gov, and cdp9@cdc.gov. Aubree J. Kelly, Bacterial Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: hr6@cdc.gov.

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