

## SEROLOGICAL EVIDENCE OF *Rickettsia parkeri* AS THE ETIOLOGICAL AGENT OF RICKETTSIOSIS IN URUGUAY

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### SUMMARY

We report three new rickettsiosis human cases in Uruguay. The three clinical cases presented clinical manifestations similar to previous reported cases of *Rickettsia parkeri* in the United States; that is mild fever (< 40 °C), malaise, headache, rash, inoculation eschar at the tick bite site, regional lymphadenopathy, and no lethality. Serological antibody-absorption tests with purified antigens of *R. parkeri* and *Rickettsia rickettsii*, associated with immunofluorescence assay indicated that the patients in two cases were infected by *R. parkeri*. Epidemiological and clinical evidences, coupled with our serological analysis, suggest that *R. parkeri* is the etiological agent of human cases of spotted fever in Uruguay, a disease that has been recognized in that country as cutaneous-ganglionic rickettsiosis.

**KEYWORDS:** Spotted fever; *Rickettsia parkeri*; Rickettsiosis; Serology; Uruguay.

### INTRODUCTION

Human rickettsiosis caused by *Rickettsia parkeri*, a spotted fever group agent, was first reported in the United States in 2004, 65 years after the first isolation of the agent from *Amblyomma maculatum* ticks in that same country<sup>12</sup>. Nowadays, *R. parkeri* is considered an emerging agent in the United States, with increasing reported cases since its official recognition as a human pathogen<sup>13</sup>. In addition, it is likely that a significant number of previously reported cases of Rocky Mountain spotted fever, presumably caused by *Rickettsia rickettsii*, were in fact caused by *R. parkeri*<sup>13,14</sup>. Besides the United States, *R. parkeri* has been reported in *Amblyomma triste* ticks from Argentina, Brazil, and Uruguay<sup>9,15,16</sup>. In this last country, a number of cases of rickettsiosis, often referred as cutaneous-ganglionic rickettsiosis, have been reported since 1990, characterized by a small papulo-nodular lesion at the tick attachment site, an influenza-like illness (fever, headache, malaise), and regional lymphadenopathy in all observed cases; some cases also presented generalized rash. No further clinical complication was observed<sup>3,4,5</sup>. Ticks associated with these cases were initially misidentified as *A. maculatum* and the rickettsial agent was prematurely identified as *R. conorii*. Subsequently, the tick species associated with rickettsiosis in Uruguay was confirmed to be *A. triste* (the closely related species *A. maculatum* is not present in Uruguay), whereas clinical and epidemiological observations have strongly indicated that the etiological agent could be *R. parkeri*, yet to be confirmed in the laboratory<sup>10,11,16</sup>. Herein, we report three new rickettsiosis cases in Uruguay, two of them with serological evidence that they were caused by *R. parkeri* after antibody absorption tests.

### CASE REPORTS

**Case 1:** A 6-year-old male child was admitted at the hospital on 07 September 2006 with fever and a cervical lymphadenopathy. One week before, he was in contact with ticks after a visit to the Hermoza Beach area, Piriápolis, southern Uruguay. In the hospital, two ticks found attached to his scalp were collected and identified as adult females of *A. triste*. Eschars were observed at the sites of tick bites on the scalp. Oral clindamycin was prescribed, but one week later generalized rash appeared on his trunk, arms, legs, and face. Large cervical lymphadenopathy was observed. Erythromycin was prescribed and no clinical abnormalities were observed in the following week. Patient's blood serum collected two weeks after onset of fever was tested for rickettsiosis through IFA for *R. parkeri*, *R. rickettsii*, *R. felis*, *R. amblyommii*, *R. rhipicephali*, and *R. bellii* antigens, as previously described<sup>7,8</sup>. Serum end-point titers to these six antigens were 2,048, 1,024, 64, 256, 512, and < 64, respectively. A second serum sample collected 30 days later was tested solely to *R. parkeri*, and *R. rickettsii*, giving the endpoint titers 2,048 and 512, respectively.

Serum cross-absorption tests were performed in order to indicate the most possible rickettsial antigen responsible for inducing infection in the human patient. For this purpose, aliquots of the first serum sample were absorbed with either *R. parkeri* or *R. rickettsii* crude antigens. Production of crude rickettsial antigens was performed following a previously described protocol<sup>1</sup>, which consisted in purifying rickettsia from infected Vero cells suspended in Minimum Essential Medium (MEM) by passage through a 25-gauge syringe needle six times to lyse the cells, sequential passage of the lysate through 5- and 2- $\mu$ m syringe filters to remove

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cellular debris, and centrifugation of the filtrates at 18,400 g at 4 °C for 20 min to pellet rickettsiae. Rickettsiae were resuspended in SFG buffer and protein concentration was determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) on rickettsiae dissolved in 100 mM of Tris, pH 7.4, and 2% sodium dodecyl sulfate. After centrifugation of 0.250 mL of purified rickettsiae, a pellet containing approximately 1.5 mg of antigen was resuspended in 1 mL of the test serum at a 1:64 dilution. The serum-antigen suspension was incubated at 37 °C for four hours followed by incubation at room temperature for 20 hours on a rocker. Thereafter, the suspension was centrifuged at 13,000 × g for 15 minutes, saving the supernatant that was the absorbed serum. Each of the absorbed sera was then tested by IFA to either *R. parkeri* or *R. rickettsii* crude antigens. If serum absorbed with one *Rickettsia* species showed no or minimal reaction against both antigens, and a strong reaction to only one of the two antigens when absorbed with the second *Rickettsia* species, this serum was considered to contain antibodies stimulated by the *Rickettsia* species (or a very closely related species) that elicited the strong reaction in the second cross-absorption and had absorbed all the antibodies in the first reaction<sup>6</sup>. After absorption with *R. rickettsii*, the serum demonstrated a 1,024 titer against *R. parkeri* and no antibodies against *R. rickettsii*, but after absorption with *R. parkeri*, there was no reaction against either of the two *Rickettsia* antigens. The anti-*Rickettsia* antibodies in this patient was considered to have been stimulated possibly by *R. parkeri*.

**Case 2:** In May 2007, a 57-year-old man from a rural area of Maldonado County, southern Uruguay, presented with fever and malaise. No tick bites were recorded within the previous few weeks. While living in the same rural area stated above, the patient had a history of recurrent fever, malaise, and tick bites during the previous two years, including an inguinal lymphadenopathy associated with fever in 2005. Patient's blood serum collected on May 2007 was tested by IFA, showing end-point titers of 2,048, 2,048, 64, 512, 128, and 256, for *R. parkeri*, *R. rickettsii*, *R. felis*, *R. amblyommii*, *R. rhipicephali*, and *R. bellii*, respectively. A second serum sample collected in July 2007, when patient presented no clinical abnormality, showed endpoint titers of 2,048, 1,024, 64, 256, 256, and 128 for *R. parkeri*, *R. rickettsii*, *R. felis*, *R. amblyommii*, *R. rhipicephali*, and *R. bellii*, respectively. Aliquots of the first serum sample were absorbed with *R. parkeri* and *R. rickettsii* antigens, as described above. After absorption with *R. rickettsii*, the serum demonstrated a 1,024 titer against *R. parkeri*, and 256 against *R. rickettsii*, but after absorption with *R. parkeri*, there was no reaction against either of the two *Rickettsia* antigens. The anti-*Rickettsia* antibodies in this patient were considered to have been stimulated possibly by *R. parkeri*.

**Case 3:** At 6 November 2005, a 38-year-old man from a rural area of Canelones County, Uruguay, presented with a cutaneous lesion on dorsum of the left foot secondary to tick bite suffered two weeks before. The lesion at the tick bite site was a small vesicula, which promptly became ulcerated and crusted, surrounded by a burnt reddish area, accompanied by fever (37-38.5 °C), painful regional lymphadenopathy, dorso-lumbar pain, and headache. Four days later, a black lesion (6-7 mm in diameter) of necrotic character was noted on the dorsum of left foot, encircled by a macular rounded area of 7-8 cm in diameter, reddish-violaceous in color, without local warm, constituted by multiple small petechial lesions (Fig.1). Edema of the foot and inflammatory lymphadenopathy at the inguino-crural zone of the same leg was also observed. On the antero-posterior areas of the trunk scarce and asymptomatic rose-colored

macular lesions, 1 cm in diameter (rash) were present. Lyme disease was first diagnosed and oral amoxicillin at 1 g per day was indicated by a 7-day period, with improvement in general symptoms. Patient's blood serum collected two weeks after onset of symptoms was tested by IFA, showing end-point titers of 4,096 to either *R. parkeri* or *R. rickettsii*, and 512 to *R. felis*. No serum cross-absorption tests were done in this case. Other laboratory findings: red blood cells sedimentation rate: 35 mm at the first hour; red blood cells count: 4,610,000 per mm<sup>3</sup>; hematocrit: 39.3%; leucocyte cells count: 7,230 per mm<sup>3</sup>; platelets: 220,000 per mm<sup>3</sup>, urine examination: normal; bacteriological examination of foot cutaneous lesion in blood agar medium: negative.



Fig. 1 - A cutaneous lesion (inoculation eschar) on dorsum of the left foot of a patient (case 3 in the text) presenting clinical cutaneous-ganglionic rickettsiosis.

## DISCUSSION

The three clinical cases of rickettsiosis reported in the present study presented clinical manifestations similar to previous reported cases due to *R. parkeri* in the United States; that is mild fever (<40 °C), malaise, headache, rash, inoculation eschar at the tick bite site, regional lymphadenopathy, and no lethality<sup>12,18</sup>. The three cases also presented similar pattern in the serological reactivities to different rickettsial antigens, characterized by higher and similar titers to *R. parkeri* and *R. rickettsii*, and substantially lower titers to other rickettsial antigens, including the spotted fever group agents *R. felis*, *R. amblyommii*, and *R. rhipicephali*. Similarly, human cases of *R. parkeri* infection in the United States showed similar IFA titers to *R. parkeri* and *R. rickettsii*<sup>13</sup>. The three patients of the present study showed clinical improvement without been treated with specific antibiotics (tetracyclines or chloramphenicol) that are usually recommended to treat rickettsiosis<sup>2</sup>. This condition is in accordance with previous reported cases of acute rickettsiosis due to *R. parkeri* in the United States, characterized by clinical manifestations that resolved even in the absence or delay treatment with specific antibiotics<sup>13</sup>.

Since no tissue sample from the patients was available in proper conditions to be tested by a direct diagnosis of rickettsia, we performed cross-absorption tests to try to indirectly identify the rickettsial agent responsible for the antibody response. In two cases, results clearly suggested that the patients were infected by *R. parkeri*. The three cases recalled previous tick infestations, but taxonomic identification of the tick was possible only in case 1, as *A. triste*. Since all three cases are from southern Uruguay, where *A. triste* is far the most common (or the only one reported) human-biting tick<sup>17</sup>, it is quite possible that the other two cases were also related to *A. triste* infestations. Interestingly, only the adult

stage of *A. triste* bites humans, and the seasonal activity of adult *A. triste* (August to February) coincides with seasonal increases in cases of human spotted fever reported in Uruguay<sup>17</sup>. All these epidemiological and clinical evidences, coupled with our serological analysis, indicate that *R. parkeri* is the possible etiological agent of human cases of spotted fever in Uruguay, a disease that has been recognized in that country as cutaneous-ganglionic rickettsiosis. We are aware that we can not assume with certainty that the illness observed on the three patients were due to an acute infection by *R. parkeri* because we did not show seroconversion between acute and convalescent sera, a crucial condition for proper serological diagnosis of acute rickettsiosis<sup>2</sup>. This information is particularly important in case 2, where patient history is more compatible with an acute rickettsial infection that might have occurred previous to the moment when serum was collected for analysis. Nonetheless, we present for the first time serological evidence of *R. parkeri* human infection in Uruguay. Finally, further studies with isolation and molecular identification of *R. parkeri* from Uruguayan patients will definitely confirm our findings. Since *R. parkeri*-infected *A. triste* ticks are also present in Argentina and Brazil, the disease will probably be diagnosed in these countries during the next years.

## RESUMO

### Evidência sorológica de *Rickettsia parkeri* como agente etiológico de rickettsiose no Uruguai

São relatados três novos casos humanos de rickettsiose no Uruguai. Os três casos clínicos apresentam manifestações clínicas semelhantes às descritas em casos de infecção por *Rickettsia parkeri* previamente relatados nos Estados Unidos, tais como: febre moderada (< 40 °C), mal-estar, cefaléia, exantema, escara de inoculação no sítio de fixação do carrapato, linfadenopatia regional e ausência de letalidade. Testes sorológicos de absorção de anticorpos com antígenos de *R. parkeri* e *Rickettsia rickettsii*, associados à reação de imunofluorescência indireta, sugerem que os pacientes de dois casos foram infectados por *R. parkeri*. Evidências clínicas e epidemiológicas, associadas com nossas análises sorológicas, sugerem que *R. parkeri* é o agente etiológico de casos humanos de febre maculosa no Uruguai, uma doença que tem sido reconhecida naquele país como rickettsiose cutâneo-ganglionar.

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