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Case Report: *Rickettsia* sp. Strain Atlantic Rainforest Infection in a Patient from a Spotted Fever-Endemic Area in Southern Brazil

Felipe S. Krawczak,[1] Sebastián Muñoz-Leal,[1] Ana Carolina Guztzazky,[2] Stefan V. Oliveira,[3] Fabiana C. P. Santos,[4] Rodrigo N. Angerami,[5] Jonas Moraes-Filho,[1] Julio C. de Souza Jr.,[2] and Marcelo B. Labruna[1]*

[1]*Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil;* [2]*Fundação Universidade Regional de Blumenau, Blumenau, Santa Catarina, Brazil;* [3]*Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, Distrito Federal, Brazil;* [4]*Instituto Adolfo Lutz, Secretaria de Estado da Saúde, São Paulo, Brazil;* [5]*Hospital das Clínicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil*

Abstract. Santa Catarina State in southern Brazil is the state with the second highest number of laboratory-confirmed cases of spotted fever illness in Brazil. However, all these cases were confirmed solely by serological analysis (seroconversion to spotted fever group rickettsiae), which has not allowed identification of the rickettsial agent. Here, a clinical case of spotted fever illness from Santa Catarina is shown by seroconversion and molecular analysis to be caused by *Rickettsia* sp. strain Atlantic rainforest. This is the third confirmed clinical case due to this emerging rickettsial agent in Brazil. Like the previous two cases, the patient presented an inoculation eschar at the tick bite site. Our molecular diagnosis was performed on DNA extracted from the crust removed from the eschar. These results are supported by previous epidemiological studies in Santa Catarina, which showed that nearly 10% of the most common human-biting ticks were infected by *Rickettsia* sp. strain Atlantic rainforest.

INTRODUCTION

Until 2009, *Rickettsia rickettsii*, the etiological agent of Rocky Mountain spotted fever (RMSF) or Brazilian spotted fever (BSF), was the only known tick-borne pathogenic *Rickettsia* known to occur in Brazil. In 2010 in the state of São Paulo, southeastern Brazil, a tick-borne spotted fever illness was reported to be caused by a novel rickettsial agent genetically related to *Rickettsia parkeri*, *Rickettsia africae*, and *Rickettsia sibirica*. This novel agent was named *Rickettsia* sp. strain Atlantic rainforest in allusion to the forest environment where the patient presumably acquired the infected tick.[1] In the subsequent year, another case of tick-borne spotted fever illness caused by a novel rickettsial agent was reported in the state of Bahia, northeastern Brazil.[2] Although the rickettsial agent of this second case was named *Rickettsia* sp. strain Bahia, further studies considered it to be the second clinical case of *Rickettsia* sp. strain Atlantic rainforest, since the two strains share identical DNA sequences.[3,4] These two cases were milder than the classical *R. rickettsii*-caused BSF cases from southeastern Brazil, and more interestingly, presented inoculation eschar (tache noire) at the tick bite site, a lesion typically described in cases of *R. parkeri* rickettsiosis in Argentina, Uruguay, and the United States, but usually not for RMSF or BSF.[5–7]

Between 2005 and 2015, a total of 314 laboratory-confirmed cases of spotted fever rickettsiosis were officially reported for the state of Santa Catarina, southern Brazil. All these cases have been confirmed by seroconversion to *R. rickettsii* antigens through analyses of acute and convalescent serum samples (unpublished official data from the Brazilian Ministry of Health). In the whole country, only São Paulo State reported more spotted fever cases than Santa Catarina; however, whereas BSF fatality rates have been around 40% in São Paulo State, no fatality has been observed in Santa Catarina.[8,9] These intriguing differences have led to suspect that the spotted fever cases of Santa Catarina were caused by a tick-borne agent different from *R. rickettsii*.[8] A recent epidemiological study suggested that at least part of the spotted fever cases of Santa Catarina was caused by *Rickettsia* sp. strain Atlantic rainforest, based on the detection of this agent in nearly 10% of the ticks collected from domestic dogs in the area where most of clinical spotted fever cases were reported.[10] Regardless, no rickettsial agent has been determined for the spotted fever cases of Santa Catarina.

CASE REPORT

In January 28, 2015, a 21-year-old woman (biology undergraduate student) was bitten by a tick on her left ankle during an ornithological expedition in a degraded fragment of Atlantic rainforest (26°51′24″S, 49°03′06″W; 21 m), Blumenau Municipality, Santa Catarina State, southern Brazil. Unfortunately, the tick was discarded after removal from the skin. Seven days after the tick bite (February 4, 2015), the patient reported a papular skin lesion at the tick bite site with intense pruritus. On February 7 (10 days after the tick bite), the patient reported headache associated with myalgia and arthralgia. On the next day, the papular lesion became surrounded by a macular rash, and symptoms of chills and fever were reported. On February 9, the patient visited a physician, who prescribed paracetamol and oral doxycycline (100 mg, every 12 hours) for 14 days. At this moment, the patient presented fever (38°C), muscle and joint pain. While fever and general pain resolved 5 days after, the skin lesion at the tick bite site evolved to a lesion with a necrotic center (inoculation eschar or tache noire), which was nearly completely healed at February 27, 30 days after the tick bite (Figure 1). Disseminated rash was not observed.

Because of suspicion of tick-borne rickettsial disease, the case was notified to the official health system of Brazil, where spotted fever is an obligate notifiable disease. Serum samples were collected at the day of the first medical visit (February 9, 2015), and 8 days later (20 days after the tick bite). The paired serum samples were tested by immunofluorescence antibody assay against crude antigens of six

*Address correspondence to Marcelo B. Labruna, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenue Professor Orlando Marques de Paiva, 87 Cidade Universitária, São Paulo, 05508-270, Brazil. E-mail: labruna@usp.br

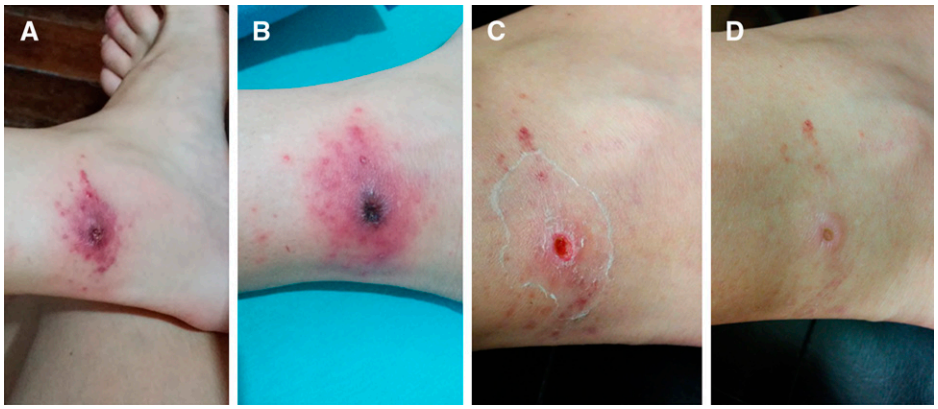

FIGURE 1. Inoculation eschar at the tick bite site on the left ankle of a patient infected with *Rickettsia* sp. strain Atlantic rainforest in Santa Catarina State, southern Brazil. (**A**) 12 days after the tick bite (DATB). (**B**) 14 DATB. (**C**) 23 DATB. (**D**) 30 DATB.

Rickettsia species from Brazil (*Rickettsia bellii*, *Rickettsia felis*, *Rickettsia amblyommii*, *Rickettsia rhipicephali*, *Rickettsia rickettsii*, and *R. parkeri*), as previously described.[10,11] Briefly, sera were diluted in 2-fold increments with phosphate-buffered saline, starting from the 1:64 dilution. Slides were incubated with fluorescein isothiocyanate–labeled goat antihuman IgG (Sigma, St. Louis, MO). For each sample, the end point IgG titer reacting with each of the six *Rickettsia* antigens was determined. Although the first serum sample did not react to any rickettsial antigen (end point titers < 64), the second serum sample had end point titers of 1,024 to *R. rickettsii*, *R. parkeri*, and *R. amblyommii*, 512 to *R. rhipicephali* and *R. bellii*, and < 64 to *R. felis.* These results indicate a seroconversion with at least 16-fold increase in titers of antibodies against *R. parkeri*, *R. rickettsii*, and *R. amblyommii*.

Twelve days after the tick bite (February 9, 2015), the patient removed the crust of the wound at the tick bite site (Figure 1) and stored it in a sterile microtube with 96% ethanol. Four months later, DNA of this skin sample was extracted using the DNAeasy Blood and Tissue Kit (Qiagen, Valencia, CA), and tested by polymerase chain reaction (PCR) with the primers Rr190.70F and Rr190.701R, targeting a ~630-bp fragment of the rickettsial *ompA* gene,[12] and by the primers 120-M59 and 120-807, targeting a ~820-bp fragment of the rickettsial *ompB* gene.[13] PCR products were purified with ExoSap (USB, Cleveland, OH) and sequenced in an ABI Prism 310 Genetic Analyzer (Applied Biosystems/Perkin Elmer, Foster City, CA). Generated sequences were shown by Basic Local Alignment Search Tool analyses (www.ncbi.nlm.nih.gov/blast) to be 100% identical to *ompA* (JQ906784) and *ompB* (GQ855236) corresponding sequences of *Rickettsia* sp. strain Atlantic rainforest. Partial DNA sequences of *ompA* (590-bp) and *ompB* (817-bp) genes generated in this study were deposited in GenBank under accession numbers KU882101 and KU882102.

DISCUSSION

A clinical case of spotted fever is confirmed by molecular analysis for the first time in southern Brazil. This is the third confirmed case of human illness due to *Rickettsia* sp. strain Atlantic rainforest. Our results are supported by previous epidemiological studies in Santa Catarina, which showed that nearly 10% of the ticks *Amblyomma ovale* and *Amblyomma aureolatum* were infected by *Rickettsia* sp. strain Atlantic rainforest, and that these two tick species were the most common human-biting ticks in the areas with reported human cases of rickettsiosis in Santa Catarina.[10,14] In addition, a recent laboratory study demonstrated the vector competence of *A. ovale* for *Rickettsia* sp. strain Atlantic rainforest.[15]

Before this study, all previous spotted fever cases in southern Brazil, including the area of this study, were confirmed solely by serological analysis (seroconversion to *R. rickettsii* antigens),[8,10] which does not allow a precise identification of the rickettsial agent, since serologic cross-reaction usually occurs between different spotted fever group *Rickettsia* species.[3] In fact, while our molecular analysis identified *Rickettsia* sp. strain Atlantic rainforest as the etiological agent, the patient seroconverted with identical end point titers to three spotted fever group agents, *R. parkeri*, *R. rickettsii*, and *R. amblyommii.* Similarly, the first report of human illness due to *Rickettsia* sp. strain Atlantic rainforest also resulted in seroconversion with similar end point titers to these three *Rickettsia* species.[1] Because previous spotted fever clinical cases of Santa Catarina[8,10] are clinically, serologically, and epidemiologically compatible with the three reported cases of *Rickettsia* sp. strain Atlantic rainforest (present study[1,2]), it is likely that this rickettsial agent is an important cause of spotted fever in southern Brazil.

Indeed, there is an urgent need for improvement of both epidemiological surveillance strategies and laboratory capacity to detect and differentiate other rickettsiosis, including mild or atypical spotted fever cases previously attributed to *R. rickettsii* based on serologic assays and not by direct tests. Here we pointed out the simplicity of collection and preservation of viable biological samples to be tested by PCR for detection of *Rickettsia* sp. strain Atlantic rainforest and other potentially pathogenic but not usually recognized *Rickettsia* species. Finally, because *Rickettsia* sp. strain Atlantic rainforest-infected *A. ovale* ticks seem to have a wide distribution in Latin America,[4] the adoption of more specific diagnostic methods could result in more human cases due to this emerging rickettsial agent in different areas of the continent.

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Authors' addresses: Felipe S. Krawczak, Sebastián Muñoz-Leal, Jonas Moraes-Filho, and Marcelo B. Labruna, Department of Preventive Veterinary Medicine, University of Sao Paulo, Sao Paulo, Brazil, E-mails: felipekvet@gmail.com, seba.munozleal@gmail.com, jonasmfilho@hotmail.com, and labruna@usp.br. Ana Carolina Guztzazky and Julio C. de Souza Jr., Biologia, Universidade Regional de Blumenau, Blumenau, Brazil, E-mails: anacarolina.gmariana@gmail.com and juliocesar_jr@terra.com.br. Stefan V. Oliveira, Ministério da Saúde, Secretaria de Vigilância em Saúde, Brasilia, Distrito Federal, Brazil, E-mail: stefanbio@yahoo.com.br. Fabiana C. P. Santos, Nucelo de Anatomica Patologica, Instituto Adolfo Lutz, Sao Paulo, Brazil, E-mail: fabianacp@yahoo.com. Rodrigo N. Angerami, Departamento de Vigilancia em Saude, Secretaria Municipal de Saude de Campinas, Campinas, Sao Paulo, Brazil, E-mail: rodrigo.angerami@gmail.com.

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