Am. J. Trop. Med. Hyg., 82(4), 2010, pp. 691–692
doi:10.4269/ajtmh.2010.09-0662

Case Report: A Patient from Argentina Infected with *Rickettsia massiliae*

Juan Carlos García-García, Aránzazu Portillo, Manuel J. Núñez, Sonia Santibáñez, Begoña Castro, and José A. Oteo*

Servicio de Medicina Interna, Hospital do Salnés, Villagarcía de Arosa, Pontevedra, Spain; Área de Enfermedades Infecciosas, Hospital San Pedro–Centro de Investigación Biomédica de La Rioja, Logroño, La Rioja, Spain

Abstract. The first confirmed case of *Rickettsia massiliae* infection in the New World (Buenos Aires, Argentina) is described. To date, only two cases of human infection had been reported in Europe. The patient, a woman, had a fever, a palpable purpuric rash on the upper and lower extremities, and a skin lesion (eschar) on the right leg compatible with tache noire. When interviewed, she reported having had contact with dog ticks. After treatment with doxycycline for 12 days, her symptoms resolved. *Rickettsia massiliae* infection was diagnosed by molecular-based detection of the microorganism in a biopsy specimen of the eschar.

Rickettsia massiliae is a spotted fever group *Rickettsia* broadly distributed around the world and is associated with *Rhipicephalus* ticks.[1–4] Its pathogenic role was suspected because its seroprevalence in humans was observed, and some authors reported that this *Rickettsia* could be the causative agent of some cases of Mediterranean spotted fever (MSF).[5] To date, only two human cases of *R. massiliae* infection in Europe have been documented and confirmed with molecular methods. The first case was detected in a blood sample from a patient diagnosed with MSF in Italy; the blood sample had been stored for 20 years before the case was detected.[6] The second case was in a patient with spotted fever and acute loss of vision in southern France.[7]

On July 1, 2005, a 56-year-old woman living in Buenos Aires, Argentina, was admitted to the emergency department of a hospital in Villagarcía de Arosa, Galicia, Spain, because of fever, chills, and malaise for 72 hours and a rash that was present for a few hours. The patient had arrived in Spain 48 hours earlier on a flight from Argentina. She had a history of convulsions related to arterio-venous malformation in the frontal brain area and was treated with phenobarbital. She had a fever (40°C) and a palpable purpuric rash all over the trunk and on the upper and lower extremities with the palms and soles affected (Figure 1).

Hemocultures were prepared and treatment was initiated with amoxicillin-clavulanic acid and an infusion of amikacin. An allergic reaction was suspected, phenobarbital was discontinued, and gabapentin treatment was initiated. Laboratory evaluation showed a leukocyte count of 1.83×10^4 cells/μL with 18% immature forms, a prothrombin ratio of 73%, an aspartate aminotransferase level of 111 IU/L, an alanine aminotransferase level of 449 IU/L, a gamma-glutamyl transpeptidase level of 1,102 IU/L, an alkaline phosphatase level of 546 U/L, and a lactate dehydrogenase level of 1,132 U/L. The patient reported the next day that she removed ticks from her dog one week earlier and found several engorged ticks in her garden. Doxycycline (100 mg every 12 hours) was then administered.

The same day, a skin lesion on the right leg compatible with tache noire was observed (Figure 2). The patient had a torpid course and a pleural effusion was developed. Treatment with prednisone was prescribed. A biopsy specimen was obtained from the lesion (eschar). On day 12, the patient was afebrile and recovered. Blood cultures and results of serologic testing for hepatitis A, B, and C viruses, Epstein-Barr virus, cytomegalovirus, *Rickettsia conorii*, *Coxiella burnetti*, *Borrellia burgdorferi*, and human immunodeficiency virus (performed with a serum sample obtained the day after her admission to hospital) were negative. The absence of antibodies against

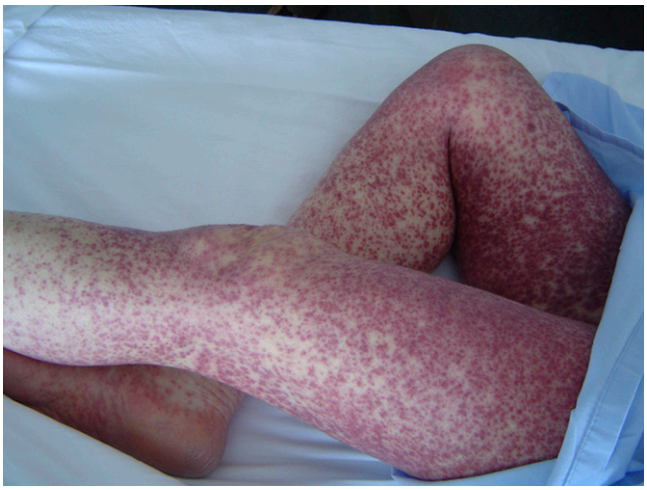

FIGURE 1. Purpuric rash on lower extremities of the patient, which affected the soles of her feet. This figure appears in color at www.ajtmh.org.

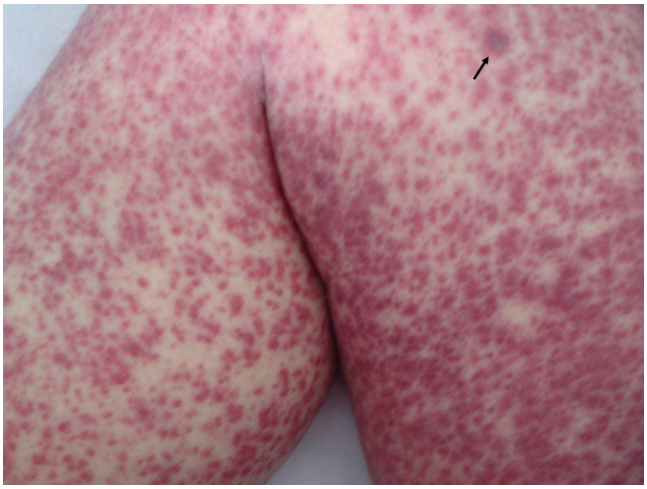

FIGURE 2. Tache noire on the right leg of the patient. This figure appears in color at www.ajtmh.org.

*Address correspondence to José A. Oteo, Área de Enfermedades Infecciosas, Hospital San Pedro–Centro de Investigación Biomédica de La Rioja, C/Piqueras 98, 26006 Logroño, La Rioja, Spain. E-mail: jaoteo@riojasalud.es

TABLE 1
Primers used for amplification of partial rickettsial genes*

Gene	Primer	Sequence (5′→3′)	Amplified fragment, basepairs	Annealing temperature (°C)	Reference
ompB	rompB OF	GTAACCGGAAGTAATCGTTTCGTAA	511	54	9
	rompB OR	GCTTTATAACCAGCTAAACCACC			
	rompB SFG IF	GTTTAATACGTGCTGCTAACCAA	420	56	9
	rompB SFG/TG IR	GGTTTGGCCCATATACCATAAG			
gltA	RpCS.877p	GGGGGCCTGCTCACGGCGG	381	48	8
	RpCS.1,258n	ATTGCAAAAAGTACAGTGAACA			
	RpCS.896p	GGCTAATGAAGCAGTGATAA	337	56	9
	RpCS.1,233n	GCGACGGTATACCCATAGC			

* *ompB* = outer membrane protein; OF = outer forward primer; OR = outer reverse primer; SFG = spotted fever group; TG = typhus group; *gltA* = citrate synthase.

R. conorii could have been the result of the antibiotic treatment, which may have reduced the antibody response.

The biopsy sample of the eschar was sent to the Department of Infectious Diseases at the Hospital San Pedro–Centro de Investigación Biomédica de La Rioja in La Rioja, Spain, because rickettsiosis was suspected. DNA was extracted from the biopsy specimen by using QIAamp Tissue Kit (Qiagen, Hilden, Germany). Presumptive diagnosis of rickettsiosis was confirmed by nested polymerase chain reaction (PCR) assays that amplified fragments of the citrate synthase (*gltA*) and outer membrane protein (*ompB*) rickettsial genes (Table 1).[8,9] Negative controls (one with template DNA but without primers and the other with primers and water instead of template DNA) and a positive control (DNA from *R. conorii* strain Malish #7) were included in all PCR assays. To minimize the potential for DNA contamination, three separate, designated areas were used for extraction of DNA and preparation of PCRs. Amplicons of the expected size were subjected to sequence determination. The nucleotide sequences obtained were found to share > 99% similarity with the corresponding *gltA* and *ompB* fragments of *R. massiliae*.

We report the third case of human *R. massiliae* infection and the first acquired in the New World (Argentina). Until recently, this *Rickettsia* species was not known to be distributed in the Americas,[4] and only two demonstrated human cases had been reported in Europe.[6,7] The potential role of this *Rickettsia* (strain Bar-29) as a human pathogen had also been suggested on the basis of the presence of antibodies against this organism in humans.[5,10] However, spotted fever group rickettsiae can cross-react, and definitive identification of the agent that stimulated the antibodies is problematic because it relies on the assumptions that all species in a region are known and that the quality of the antigens used for each species is ideal.

Our findings extend the role of *R. massiliae* as an etiologic agent of tick-borne spotted fever rickettsiosis in the New World, where it had been previously detected in *R. sanguineus*, a tick species widely distributed around the world.

Received November 4, 2009. Accepted for publication December 26, 2009.

Acknowledgments: We thank Fátima Bacellar, Rita de Sousa, and Natacha Milhano (Centro de Estudos de Vectores e Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal) for supplying spotted fever group *Rickettsia*-positive controls. The American Committee on Clinical Tropical Medicine and Travelers' Health (ACCTMTH) assisted with publication expenses.

Financial support: This study was supported in part by a grant from the Instituto de Salud Carlos III (EMER 07-033), Ministerio de Ciencia e Innovación, Spain.

Authors' addresses: Juan Carlos García-García, Manuel J. Núñez, and Begoña Castro, Servicio de Medicina Interna, Hospital do Salnés, Ande Rubianes s/n, 36600 Villagarcía de Arosa, Pontevedra, Spain, E-mails: juan.carlos.garcia.garcia@sergas.es, majenu@cmpont.es, and begona.castro.paredes@sergas.es. Aránzazu Portillo, Sonia Santibáñez, and José A. Oteo, Área de Enfermedades Infecciosas, Hospital San Pedro, Centro de Investigación Biomédica de La Rioja, C/Piqueras 98, 26006 Logroño, La Rioja, Spain, E-mails: aportillo@riojasalud.es, ssantibanez@riojasalud.es, and jaoteo@riojasalud.es.

REFERENCES

1. Beati L, Raoult D, 1993. *Rickettsia massiliae* sp. nov., a new spotted fever group *Rickettsia*. *Int J Syst Bacteriol 43:* 839–840.
2. Tissot Dupont H, Cornet JP, Raoult D, 1994. Identification of rickettsiae from ticks collected in the Central African Republic using the polymerase chain reaction. *Am J Trop Med Hyg 50:* 373–380.
3. Oteo JA, Portillo A, Santibáñez S, Pérez-Martínez L, Blanco JR, Jiménez S, Ibarra V, Pérez-Palacios A, Sanz M, 2006. Prevalence of spotted fever group *Rickettsia* species detected in ticks in La Rioja, Spain. *Ann NY Acad Sci 1078:* 320–323.
4. Eremeeva M, Bosserman EA, Demma LJ, Zambrano LM, Blau DM, Dasch G, 2006. Isolation and identification of *Rickettsia massiliae* from *Rhipicephalus sanguineus* ticks collected in Arizona. *Appl Environ Microbiol 72:* 5569–5577.
5. Cardeñosa N, Segura F, Raoult D, 2003. Serosurvey among Mediterranean spotted fever patients of a new spotted fever group rickettsial strain (Bar29). *Eur J Epidemiol 18:* 351–356.
6. Vitale G, Mansueto S, Rolain JM, Raoult D, 2006. *Rickettsia massiliae* human isolation. *Emerg Infect Dis 12:* 174–175.
7. Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, Labauge P, Raoult D, 2008. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis 2:* e338.
8. Regnery RL, Spruill CL, Plikaytis BD, 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol 173:* 1576–1589.
9. Choi YJ, Jang WJ, Ryu JS, Lee SH, Park KH, Paik HS, Koh YS, Choi MS, Kim IS, 2005. Spotted fever group and typhus group rickettsioses in humans, South Korea. *Emerg Infect Dis 11:* 237–244.
10. Bernabeu-Wittel M, del Toro MD, Nogueras MM, Muniain MA, Cardeñosa N, Segura F, Pachon J, 2006. Presence of human past infections due to the Bar29 rickettsial strain in southern Spain. *J Infect 52:* e117–e119.