Am. J. Trop. Med. Hyg., 77(2), 2007, pp. 376–377

Short Report: Detection of *Rickettsia africae* in *Rhipicephalus* (*Boophilus*) *decoloratus* Ticks from the Republic of Botswana, South Africa

Aránzazu Portillo, Laura Pérez-Martínez, Sonia Santibáñez, José R. Blanco, Valvanera Ibarra, and José A. Oteo*
Área de Enfermedades Infecciosas, Hospital San Pedro-Centro de Investigacíon Biomédica de La Rioja (CIBIR), Logroño, Spain

Abstract. A total of 53 engorged adult ticks belonging to the species *Rhipicephalus* (*Boophilus*) *decoloratus* (N = 9), *Rhipicephalus evertsi evertsi* (N = 27), *Rhipicephalus appendiculatus* (N = 9), *Amblyomma hebraeum* (N = 5), and *Hyalomma marginatum turanicum* (N = 3), were removed from oryx in Botswana (South Africa). They were tested for the presence of spotted fever group (SFG) *Rickettsia* and *Anaplasma phagocytophilum* using polymerase chain reaction (PCR). Seventy-seven percent of *R. decoloratus* as well as twenty percent of *A. hebraeum* were positive for *ompA*, *gltA* and 16S rRNA SFG *Rickettsia* PCR assays. All nucleotide sequences were homologous to *Rickettsia africae*, the agent of African tick-bite fever (ATBF). None of the tested ticks was positive for 16S rRNA *A. phagocytophilum* PCR assays. These results suggest for the first time that *R. decoloratus* ticks may be reservoirs of *R. africae*, and support the ATBF risk in this area.

Natural disasters, wars, globalization of business, international adoption, immigration, and tourism have increased the risk of infectious diseases. Spotted fever group (SFG) rickettsioses are important emerging tick-borne human infections worldwide.[1–3] Among them, African tick bite fever (ATBF), caused by *Rickettsia africae*, is currently the most common rickettsioses in sub-Saharan Africa.[4,5] This infection must be taken into account in the differential diagnosis of patients with fever and/or exanthema returning from South Africa. In Spain, three cases of ATBF confirmed by polymerase chain reaction (PCR) were recently reported in tourists returning from South Africa.[6] Several tick species have been found to be vectors of SFG rickettsiae in Africa.[7–9] However, little is known about the prevalence of SFG *Rickettsia* in vector ticks from Botswana. The aim of our study was to detect the presence of SFG *Rickettsia* and *Anaplasma phagocytophilum* in ticks obtained in Botswana using PCR and sequence analysis. A total of 53 adult ticks belonging to the species *Rhipicephalus* (*Boophilus) decoloratus* (N = 9), *Rhipicephalus evertsi evertsi* (N = 27), *Rhipicephalus appendiculatus* (N = 9), *Amblyomma hebraeum* (N = 5), and *Hyalomma marginatum turanicum* (N = 3) were removed from oryx (*Oryx gacella*) in the Republic of Botswana, near Limpopo River. All examined ticks were engorged. Briefly, each tick was disinfected by immersion in alcohol 70% before being processed. They were rinsed in distilled water and dried on sterile filter paper under a laminar flow hood. Each tick was crushed using a sterile pestle, and its DNA was extracted individually using the QIAamp Tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Semi-nested PCR amplifications for the SFG rickettsial 190-kd surface antigen gene (*ompA*) using the primer pairs Rr190-70p and Rr190-701n for the primary reaction, followed by Rr190-70p and Rr190-602n in the second run were perfomed. Non-nested PCR assays for rickettsial *gltA* (using primer pair RpCS.877p and RpCS.1258n) and 16S rRNA genes (with primers fD1 and Rc16S.452n) were also carried out.[10] Furthermore, PCR primers ge9f and ge10r, which amplify a portion of *A. phagocytophilum* 16S rRNA gene, were used.[11] PCR products were purified and sequenced. Nucleotide sequence homology searches were made at the network server of the National Center for Biotechnology Information (NCBI) using BLAST.

PCR with 16S rRNA primers of the 53 studied ticks did not show DNA of *A. phagocytophilum.* However, *R. decoloratus* and *A. hebraeum* specimens were positive for *ompA*, *gltA,* and 16S rRNA rickettsial PCR assays. The rate of rickettsial-positive genes identification in *R. decoloratus* ticks was higher (77%) than that in *A. hebraeum* ticks (20%).

The sequences of *ompA* PCR products (483–488 bp) obtained from *R. decoloratus* and *A. hebraeum* were 100% similar to that of *R. africae* (GenBank accession number U43790). The nucleotide sequences obtained from the *gltA* PCR products (322–339 bp) were closest to *Rickettsia africae,* sharing 99.7–100% identity with the partial sequence of the *gltA* gene (GenBank accession number U59733). The sequences of 16S rRNA PCR amplicons (377–407 bp) showed 99.2–100% similarity with *Rickettsia africae* (GenBank accession number L36098).

Our study showed the presence of *R. africae* in *A. hebraeum* and *R. decoloratus* ticks collected from oryx in Botswana. Up to our knowledge, this is the first time that this *Rickettsia* species is found in *R. decoloratus*. However, the removal of the ticks from oryx suggests the possible presence of *R. africae* in the blood meal. Because *Rhipicephalus* ticks were removed from animals, they could simply be passive carriers. More studies are needed to show if *R. decoloratus* ticks are able to maintain the rickettsiae and to transmit them experimentally. This tick species is the most abundant one in the region, according to a survey of free-living ixodid ticks in Limpopo Province.[12] Our results support the ATBF risk in the area. Additional surveillance is needed to increase physician and public awareness of the potential risk of disease to humans from exposure to this tick.

Received December 27, 2006. Accepted for publication May 8, 2007.

Acknowledgments: We are very grateful to Agustín Estrada-Peña and José Manuel Venzal (University of Zaragoza, Spain) for their contribution to the classification of African ticks.

Financial support: This study was partly supported by grants from the Fondo de Investigación Sanitaria (G03/057 and PI051460), Ministerio de Sanidad y Consumo, Spain.

* Address correspondence to José A. Oteo, Área de Enfermedades Infecciosas, Hospital San Pedro. C/Piqueras, 98-7ª N.E.; 26006 Logroño (La Rioja), Spain. E-mail: jaoteo@riojasalud.es

Authors' addresses: Aránzazu Portillo, Laura Pérez-Martínez, Sonia Santibáñez, José R. Blanco, Valvanera Ibarra, and José A. Oteo,

Área de Enfermedades Infecciosas, Hospital San Pedro–Centro de Investigacíon Biomédica de La Rioja (CIBIR), C/Piqueras, 98; 26006 Logroño (La Rioja), Spain.

REFERENCES

1. Hechemy KE, Oteo JA, Raoult D, Silverman DJ, Blanco JR, 2005. New insights into rickettsioses: genomics, proteomics, pathobiology, and the international threat of rickettsial diseases: Introduction. *Ann N Y Acad Sci 1063:* xiii.
2. Parola P, Paddock CD, Raoult D, 2005. Tick-borne rickettsioses around the World: emerging diseases challenging old concepts. *Clin Microbiol Rev 18:* 719–756.
3. Ndip LM, Fokam EB, Bouyer DH, Ndip RN, Titanji VPK, Walker DH, Mc Bride JW, 2004. Detection of *Rickettsia africae* in patients and ticks along the coastal region of Cameroon. *Am J Trop Med Hyg 71:* 363–366.
4. Raoult D, Fournier PE, Fenollar F, Jensenius M, Prioe T, de Pina JJ, Caruso G, Jones N, Laferl H, Rosenblatt JE, Marrie TJ, 2001. *Rickettsia africae*, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med 344:* 1504–1510.
5. Jensenius M, Fournier PE, Kelly P, Myrvang B, Raoult D, 2003. African tick bite fever. *Lancet Infect Dis 3:* 557–564.
6. Oteo JA, Portillo A, Blanco JR, Ibarra V, Santibáñez S, 2004. *Rickettsia africae* infection. Three cases confirmed by PCR. *Med Clin (Barc) 122:* 786–788.
7. Parola P, 2006. Rickettsiosis in Sub-Saharan Africa. *Ann N Y Acad Sci 1078:* 42–47.
8. Parola P, Inokuma H, Camicas JL, Brouqui P, Raoult D, 2001. Detection and identification of spotted fever group *Rickettsiae* and *Ehrlichiae* in African ticks. *Emerg Infect Dis 7:* 1014–1017.
9. Macaluso KR, Davis J, Alam U, Korman A, Rutherford JS, Rosenberg R, Azad AF, 2003. Spotted fever group *Rickettsiae* in ticks from the Masai Mara region of Kenya. *Am J Trop Med Hyg 68:* 551–553.
10. Venzal JM, Portillo A, Estrada-Peña A, Castro O, Cabrera PA, Oteo JA, 2004. *Rickettsia parkeri* in *Amblyomma triste* from Uruguay. *Emerg Infect Dis 10:* 1493–1495.
11. Portillo A, Santos AS, Santibáñez S, Pérez-Martínez L, Blanco JR, Ibarra V, Oteo JA, 2005. Detection of a non-pathogenic variant of *Anaplasma phagocytophilum* in *Ixodes ricinus* from La Rioja, Spain. *Ann N Y Acad Sci 1063:* 333–336.
12. Schroder B, Uys AC, Reilly BK, 2006. A survey of free living ixodid ticks on a commercial game farm in the Thabazimbi District, Limpopo Province, South Africa. *J S Afr Vet Assoc 77:* 141–144.