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

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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 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 performed. 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* *ompA* 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. africace* 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.

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