Ticks and Tick-borne Diseases 3 (2012) 376–378

Contents lists available at SciVerse ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Rickettsia africae in *Hyalomma dromedarii* ticks from sub-Saharan Algeria

Tahar Kernif[a,b], Amel Djerbouh[a], Oleg Mediannikov[b], Bouhous Ayach[c], Jean-Marc Rolain[b], Didier Raoult[b], Philippe Parola[b], Idir Bitam[a,*]

[a] *Service d'Ecologie et des Systèmes Vectoriels, Institut Pasteur d'Algérie, Algiers, Algeria*
[b] *Aix Marseille Université, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63, CNRS 7278, IRD 198, Inserm 1095, WHO Collaborative Center for Rickettsioses and Other Arthropod Borne Bacterial Diseases, Faculté de Médecine, 27 bd Jean Moulin, 13385 Marseille cedex 5, France*
[c] *Direction des Services Agricoles de la Wilaya d'Adrar, Algeria*

ARTICLE INFO

Keywords:
Rickettsia africae
Hyalomma dromedarii ticks
Algeria

ABSTRACT

Spotted fever group (SFG) rickettsioses are caused by obligate, intracellular Gram-negative bacteria of the genus *Rickettsia*. In recent years, several species and subspecies of rickettsias have been identified as emerging pathogens throughout the world, including sub-Saharan Africa. We report here the detection of *Rickettsia africae*, the agent responsible for African tick-bite fever, by amplification of fragments of *gltA* and *ompA* genes and multi-spacer typing from *Hyalomma dromedarii* ticks collected from the camel *Camelus dromedarius* in the Adrar and Béchar region (sub-Saharan Algeria). To date, *R. africae* has been associated mainly with *Amblyomma* spp. The role of *H. dromedarii* in the epidemiology of *R. africae* requires further investigation.

Introduction

Spotted fever group (SFG) rickettsioses are an important group of emerging, worldwide occurring tick-borne human infections. Clinical signs generally include fever, headache, rash, and sometimes eschar formation at the site of the tick bite. To date, 6 pathogenic, tick-borne spotted fever group rickettsias are found in sub-Saharan Africa. These include *Rickettsia conorii conorii*, *R. conorii caspia*, *R. africae*, *R. aeschlimannii*, *R. sibirica mongolitimonae*, and *R. massiliae* (Cazorla et al., 2008; Parola et al., 2005). The aim of this work was to detect spotted fever group (SFG) rickettsiae in ticks from 2 unexplored regions of sub-Saharan Algeria.

Materials and methods

From February to May 2008, a total of 183 ticks was collected from the camel species *Camelus dromedarius* in Adrar (27°51′N, 0°19′W), and 20 ticks were collected in Béchar (31°34′N, 2°16′W). Both regions are situated in sub-Saharan Algeria. The tick collection was from camels from 3 oases in the region of Adrar (Timiaouine, Adrar, and Bordj Badji Mokhtar) and one oase region in Béchar (Fig. 1). The ticks were kept in 70% ethanol and sent to the WHO Collaborative Centre for Rickettsial Diseases and Other Arthropod-borne Bacterial Diseases (Marseille, France). Phenotypic identification of the ticks was made with current taxonomic criteria (Walker et al., 2003).

DNA from 218 ticks as well as negative controls from non-infected lice colonies in our laboratory were extracted using a QIAmp Tissue Kit (QIAGEN, Hilden, Germany), as previously described. DNAs were subjected to real-time quantitative PCR (qPCR) with *Rickettsia*-specific primers and Taqman probes targeting a partial sequence of the citrate synthase *gltA* (RKND03 system) in an Applied system instrument "7900 HT" (Varagnol et al., 2009). The positive control consisted of DNA from *R. montanensis* (qPCR). Ticks samples are recorded as positive when the cycle threshold was Ct≤30. Positive samples from the qPCR were confirmed by PCR amplification and direct sequencing of *ompA* and *gltA* genes, as previously described (Sarih et al., 2008). Additionally, 2 multi-spacer typings (MST) of *Rickettsia* sp. were used, *dksA-xerC* and *rpmE-tRNA^{fmet}*, in DNA samples found to be positive for *R. africae* (Fournier and Raoult, 2007). Finally, identification of ticks that tested positive for *Rickettsia* was performed using molecular tools for PCR amplification and sequencing of fragments based on mitochondrial 12S rDNA gene using T1B and T2A primers (Beati and Keirans, 2001). All obtained sequences were assembled, edited, and compared to those available in GenBank.

DNA sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM, PE Applied Biosystems, USA). All obtained sequences were analyzed using BLAST (www.ncbi.nlm.nih.gov/BLAST) and compared with *Rickettsia* sequences available in GenBank.

* Corresponding author. Tel.: +213 21 673365; fax: +213 21 673365; mobile: +213 71 995693.
E-mail address: idirbitam@gmail.com (I. Bitam).

1877-959X/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ttbdis.2012.10.013

Table 1
PCR detection and sequence identification of spotted fever group *Rickettsia* spp. from ticks collected in sub-Saharan Algeria.

Tick species (no. of tested specimens)	Species of *Rickettsia* detected (no. of PCR-positives)	Rickettsial gene target							
		ompA		*gltA*		MST			
						dksA-xerC		*rpmE-tRNA^{fmet}*	
		S (%)	GB no.	S (%)	GB no.	S (%)	GB no.	S (%)	GB no.
Hyalomma dromedarii (*n* = 139)	*R. africae* (*n* = 6)	99.2	CP001612.1	100	CP001612.1	100	HQ335138.1	100	CP001612.1
H. impeltatum (*n* = 59)	None (*n* = 0)								
H. truncatum (*n* = 5)	None (*n* = 0)								

S (%), similarity; GB no., GenBank accession number; MST, multi-spacer typing.

Results

According to current taxonomic criteria, 119 ticks from Adrar were identified as *Hyalomma dromedarii*, 59 were identified as *H. impeltatum*, and 5 were identified as *H. truncatum*. All ticks collected in Béchar were morphologically identified as *H. dromedarii*. Using qPCR, rickettsial DNA was detected in 6/203 (3.0%) ticks (5 from Adrar and 1 from Béchar), including 6/139 *H. dromedarii* (4.3%). All positive ticks tested positive by 2 standard qPCR amplifications using *ompA* and *gltA* primers. Sequence analyses for all *H. dromedarii* samples showed 99.2% similarity with the fragment of the *ompA* sequence and 100% similarity with the *gltA* sequence of *R. africae* (GenBank accession no. CP001612.1). All samples positive for *R. africae* were used for MST typing. Sequencing of PCR-amplified *dksA-xerC* and *rpmE-tRNA^{fmet}* gave a sequence with 100% similarity to the sequence of *R. africae* detected in *H. dromedarii* ticks collected from Egypt (GenBank accession no. HQ335138.1) and the published genomic *R. africae* sequence in GenBank (CP001612.1), respectively (Table 1). Additionally, 6 ticks positive for *R. africae* were identified through PCR of the tick mitochondrial 12S rDNA gene. Sequence analysis showed 100% similarity to the corresponding 12S rDNA of *H. dromedarii* (GenBank accession no. AF150036.1).

Discussion

We report here the first detection of *R. africae*, the agent of African tick bite fever (ATBF), in Algeria. The first human case of ATBF was described in 1992, and the disease is considered the most common rickettsiosis in sub-Saharan Africa (Cazorla et al., 2008).

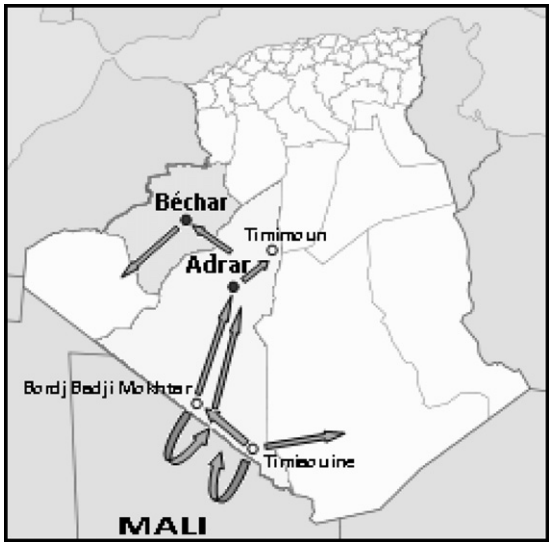

Fig. 1. Sites where tick collections were done.

Hundreds of cases have been reported, mainly in returned travelers (Cazorla et al., 2008). However, ATBF was reported also from indigenous patients in Cameroon in 2004, as documented by serology and PCR (Ndip et al., 2004).

R. africae has been detected by PCR in ticks from many sub-Saharan African countries, including Niger, Mali, Sudan, Senegal, Burundi, and South Africa (Mediannikov et al., 2010a; Parola et al., 2001; Portillo et al., 2007). The geographic distribution of *R. africae* parallels the geographic distribution of the usually recognized vector and reservoir, *Amblyomma* ticks. Among this genus, *A. hebraeum* is the most common vector of *R. africae* in South Africa, and *A. variegatum* is the predominant vector for the rest of sub-Saharan Africa (Cazorla et al., 2008). *R. africae* has seldom been detected in other tick genera including *Rhipicephalus* (*Boophilus*) *decoloratus* removed from oryx (*Oryx gacella*) in South Africa (Portillo et al., 2007), *Rhipicephalus evertsi evertsi* and *Rhipicephalus* (*Boophilus*) *annulatus* in Senegal (Mediannikov et al., 2010b). This does not imply that these ticks are vectors of *R. africae*, as ticks may have fed on bacteremic animals. Here, we report the detection of *R. africae* in *H. dromedarii* collected on camels (*C. dromedarius*). The same genotype of *R. africae* was recently identified in *H. dromedarii* in Egypt (Abdel-Shafy et al., 2012). Autochthonous camels in the Timiaouin and Bordj Badji mokhtar oases move to other regions, including Béchar. In addition, during periods of drought, the breeders move their camels to the oases of northern Mali (Fig. 1). Identification of *H. dromedarii* as a potential vector of *R. africae* is pending. These oases are often visited by tourists, and *H. dromedarii* may also bite humans.

In conclusion, our result questions the association between *H. dromedarii* and *R. africae* and the potential role of the tick as a vector for ATBF in North Africa. Therefore, the role of *H. dromedarii* in the epidemiology of *R. africae* requires further investigation. Finally, clinicians in Algeria or in those countries that treat persons returning from these oases should be on alert for these SFG rickettioses. Entomological surveys will allow a better understanding of tick-borne spotted fever and will help to highlight the epidemiological aspects of SFG rickettsioses in Algeria.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

We are grateful to the IRD jeune équipe d'Algérie (associated with the IRD 198, Marseille) for their technical assistance.

References

Abdel-Shafy, S., Allam, N.A., Mediannikov, O., Parola, P., Raoult, D., 2012. Molecular detection of spotted fever group rickettsiae associated with ixodid ticks in Egypt. Vector Borne Zoon. Dis. 12, 346–359.

Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J. Parasitol. 87, 32–48.

Cazorla, C., Socolovschi, C., Jensenius, M., Parola, P., 2008. Tick-borne diseases: tick-borne spotted fever rickettsioses in Africa. Infect. Dis. Clin. N. Am. 22, 531–544.

Fournier, P.E., Raoult, D., 2007. Identification of rickettsial isolates at the species level using multi-spacer typing. BMC Microbiol. 7, 72.

Mediannikov, O., Diatta, G., Fenollar, F., Sokhna, C., Trape, J.F., Raoult, D., 2010a. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. PLoS Negl. Trop. Dis., 4.

Mediannikov, O., Trape, J.F., Diatta, G., Parola, P., Fournier, P.E., Raoult, D., 2010b. *Rickettsia africae*, Western Africa. Emerg. Infect. Dis. 16, 571–573.

Ndip, L.M., Bouyer, D.H., Travassos Da Rosa, A.P., Titanji, V.P., Tesh, R.B., Walker, D.H., 2004. Acute spotted fever rickettsiosis among febrile patients, Cameroon. Emerg. Infect. Dis. 10, 432–437.

Parola, P., Inokuma, H., Camicas, J.L., Brouqui, P., Raoult, D., 2001. Detection and identification of spotted fever group Rickettsiae and Ehrlichiae in African ticks. Emerg. Infect. Dis. 7, 1014–1017.

Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev. 18, 719–756.

Portillo, A., Perez-Martinez, L., Santibanez, S., Blanco, J.R., Ibarra, V., Oteo, J.A., 2007. Detection of *Rickettsia africae* in *Rhipicephalus* (*Boophilus*) *decoloratus* ticks from the Republic of Botswana, South Africa. Am. J. Trop. Med. Hyg. 77, 376–377.

Sarih, M., Socolovschi, C., Boudebouch, N., Hassar, M., Raoult, D., Parola, P., 2008. Spotted fever group rickettsiae in ticks, Morocco. Emerg. Infect. Dis. 14, 1067–1073.

Varagnol, M., Parola, P., Jouan, R., Beaucournu, J.C., Rolain, J.M., Raoult, D., 2009. First detection of *Rickettsia felis* and *Bartonella clarridgeiae* in fleas from Laos. Clin. Microbiol. Infect. 15 (Suppl. 2), 334–335.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., 2003. Ticks of Domestic Animals in Africa. Edinburgh, Scotland, Bioscience Reports.