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Molecular survey for spotted fever group rickettsiae in ticks from Morocco

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INTRODUCTION

The number of representatives of the genus *Rickettsia* and the number of newly described rickettsioses have increased in recent decades as a result of improved cell culture isolation techniques and of the extensive use of bacterial detection and identification based on molecular biology techniques [1]. Comparison of the sequences of PCR-amplified fragments of rickettsial genes has become a reliable method for the identification of rickettsiae in arthropods, including ticks. As a consequence, from 1984 to 2008, more than a dozen rickettsial species or subspecies were identified as emerging agents of tick-borne rickettsioses throughout the world. As an illustration, in Morocco five rickettsial species, all human pathogens, have been identified from ticks in the Taza region, including: *Rickettsia massiliae* in *Rhipicephalus sanguineus*, *R. slovaca* and *R. raoultii* in *Dermacentor marginatus*, *R. aeschlimannii in Hyalomma marginatum*, and finally *R. monacensis* and *R. helvetica* in *Ixodes ricinus* [2]. We present here the detection and characterisation of rickettsiae in more ticks collected in different areas of Morocco using PCR and sequence analysis of amplified products.

MATERIAL AND METHODS

From May to August 2007, ticks were collected from animals and on vegetation in five different regions of Morocco: Casablanca, Taza, Marrakech, Meknes and Kenitra. All ticks were adults and were morphologically identified by one of us (SM) using the usual taxonomic keys to the species or genus level. DNA was extracted by using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Rickettsial DNA was detected by PCR using primers *Rp* CS.409*p* and *Rp* CS.1258*n*, which amplify a 750-bp fragment of the citrate synthase gene (*gltA*) of *Rickettsia* [3]. Additionally, all positive ticks for *gltA* were tested for the *ompA* gene of *Rickettsia* using primers Rr. 190.70 and Rr. 190.701, which amplify a 629- to 632-bp fragment [3]. A negative control with distilled water instead of tick DNA template in the PCR mixture and a positive control (DNA from *R. montanensis*) were included in each test. DNA samples were transported to Marseille, France, where all PCR reactions were carried out. PCR products were purified and sequencing was performed as previously described [2,3]. All obtained sequences were assembled and edited with Auto Assembler software (version 1.4; Perkin-Elmer, Courtaboeuf, France). Sequences were analysed by BLAST sequencing analysis of the sequences in the GenBank database.

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RESULTS

A total of 167 specimens representing four species and two genera of ticks were collected, including *Rh. sanguineus* (125 specimens), *Rh. sanguineus* group female (15 specimens), *Rh. bursa* (10 specimens), *Rh. turanicus* (three specimens) and *Ixodes ricinus* (14 specimens) (Table 1). Using a *glt*A PCR, rickettsial DNA was detected in eight (4.8%) of the 167 ticks. Four (26.7%) of 15 *Rh. bursa* collected in the Kenitra region contained rickettsia DNA exhibiting a nucleotide sequence of the r*Omp*A gene fragment that was 99.8% similar to *R. massiliae*. Three (21.4%) of 14 *I. ricinus* collected in the Taza region contained rickettsia DNA exhibiting a nucleotide sequence of the r*Omp* A gene fragment that was 100% similar to *R. monacensis*. Finally, one (2.5%) of the 40 *Rh. sanguineus* collected in the Casablanca region contained rickettsia DNA exhibiting a nucleotide sequence of the r*Omp* A gene fragment that was 99.4% similar to *R. conorii* (Table 1).

DISCUSSION

In Morocco, Mediterranean spotted fever due to *R. conorii* has been the sole human tick-borne rickettsiosis known by clinicians, although

Table 1. Detection and identification of spotted fever group *Rickettsia* spp. from ticks collected in Morocco by the polymerase chain reaction (PCR) and sequencing

Tick species (number of tested specimens)	Region and number of the ticks collected	Number of ticks positive by PCR */total examined	Identification by gene sequence	GenBank accession number	Identities
Ixodes ricinus (14)	Taza: 14	3/14 Taza	*R. monacensis*	DQ157778	100%
Rhipicephalus sanguineus (125)	Casablanca: 40	1/40 Casablanca	*R. conorii*	AE008674	99.4%
	Kenitra: 22	———			
	Marrackech: 33	———			
	Meknes: 30	———			
Rh. bursa (10)	Meknes: 10	0/10 Kenitra	–	–	–
		———			
Rh. sanguineus group (15)	Kenitra: 15	4/15 Kenitra	*R. massiliae*	U43799	99.8%
		———			
Rh. turanicus (3)	Kenitra: 3	———	———	———	
		———	———	———	

*Ticks positive for *gltA* PCR were tested for the *OmpA* gene, and the amplified fragments of the gene were sequenced.

tick-borne rickettsial agents other than *R. conorii conorii* have been recently detected in ticks. Although *R. sanguineus* is the well-known vector of *R. conorii conorii*, the agent of MSF, this is the first time to our knowledge that this rickettsia is detected by molecular methods in ticks from Morocco. This work provides also the first detection of *R. massiliae* in ticks from other regions than Taza in Morocco, like Kenitra. This emerging pathogen [4] has been associated with several species of *Rhipicephalus* spp., including those of the *Rh. sanguineus* group, some of which are vectors but also reservoirs of the bacteria [5]. Finally, the detection of *R. monacensis* in the Taza areas initiated in 2006 confirms the presence of this emerging pathogen in this humid area of the middle occidental Atlas, which is the only site in Morocco that has revealed the presence of *I. ricinus* ticks.

Our findings extend the knowledge of the geographic distribution of SFG rickettsiae in Morocco. Still further studies are needed to determine the epidemiological and clinical importance of the different rickettsioses in this region.

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