Rickettsia africae and *Candidatus Rickettsia barbariae* in Ticks in Israel

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Abstract. DNA of several spotted fever group rickettsiae was found in ticks in Israel. The findings include evidence for the existence of *Rickettsia africae* and *Candidatus Rickettsia barbariae* in ticks in Israel. The DNA of *R. africae* was detected in a *Hyalomma detritum* tick from a wild boar and DNA of *C. Rickettsia barbariae* was detected in *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* collected from vegetation. The DNA of *Rickettsia massiliae* was found in *Rh. sanguineus* and *Haemaphysalis erinacei*, whereas DNA of *Rickettsia sibirica mongolitimonae* was detected in a *Rhipicephalus (Boophilus) annulatus*. Clinicians should be aware that diseases caused by a variety of rickettsiae previously thought to be present only in other countries outside of the Middle East may infect residents of Israel who have not necessarily traveled overseas. Furthermore, this study reveals again that the epidemiology of the spotted fever group rickettsiae may not only involve *Rickettsia conorii* but may include other rickettsiae.

INTRODUCTION

Bacteria belonging to the genus *Rickettsia* are obligate intracellular, gram-negative coccobacillae. *Rickettsia conorii israelensis*, the etiological agent of Israel spotted fever was first discovered in Israel in 1943¹; since then molecular evidences for the existence of other pathogenic rickettsial species, such as *Rickettsia aeschlimannii*, *Rickettsia massiliae*, *Rickettsia sibirica mongolitimonae*,^{2–4} and *Rickettsia felis*,⁵ and recently *R. africae*² have been found in Israel. With the exception of flea-borne *R. felis*, the main vectors of the spotted fever group (SFG) rickettsiae are ticks that also serve as reservoirs. *Rhipicephalus sanguineus* and probably *Rhipicephalus turanicus* are the vectors of *R. conorii*, which is endemic in Israel.^{6,7}

Israel's central position in Asia between Africa and Europe makes it an ideal location for examining the possibility of the introduction of different rickettsial species between these geographical regions. Ticks can be transferred from one continent to another by birds and animal migrations and therefore the importance of determining the geographical distribution of the different rickettsial species in this region.

The polymerase chain reaction (PCR) method, as used in this study, has become a powerful tool for the identification of rickettsial species and their spread to different parts of the world.^{3,4} In this study, ticks from animals and vegetation were collected to expand our database and knowledge of ticks that may be reservoirs and vectors for transmitting rickettsiae in Israel.

MATERIALS AND METHODS

Three hundred and sixty-two ticks were collected in 2010 either while infesting wild and domestic animals or questing on plants. Animal species from which ticks were picked included: Mesopotamian fallow deer (*Dama mesopotamica*), ibex (*Capra ibex*), honey badger (*Mellivora capensis*), hedgehog (*Erinaceus concolor*), addax (*Addax nasomaculatus*), golden jackal (*Canis aureus*), wild boar fox (*Sus srofa*), dog

(*Canis familiaris*), domestic cat (*Felis catus*) and rabbit (*Oryctolagus cuniculus*). Ticks were collected also by flagging from vegetation in two sites in central Israel (Hulda and Caesarea). Collected ticks were stored in 70% ethanol and later identified using standard taxonomic keys.^{8–11}

Ticks were stored in 70% ethanol and DNA was extracted using the QIAamp Mini kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions. Tick extracts were tested for SFG rickettsae by nested PCR to amplify a fragment of 17 kDa protein antigen gene as described previously.¹² Species identification of SFG rickettsiae was done by sequencing of 70–602 nucleotide fragment of the outer membrane protein A (OmpA) and BLAST (basic local alignment tool) analysis as described previously.⁴ All new sequences generated during this study were submitted to National Center for Biotechnology Information (NCBI) GenBank under the following accession nos.: *C. Rickettsia barbariae* - JF700253, *R. africae* - JF700254, *R. sibirica mongolitimonae* - JF700255, and *R. massiliae* - KJ187075-187077.

RESULTS

Overall, 99 *Rhipicephalus (Boophilus) annulatus*, 185 *Rh. turanicus*, 57 *Rh. sanguineus*, 3 *Hyalomma marginatum*, 7 *Haemaphysalis erinacei*, 4 *Hyalomma detritum* and 7 *Hyalomma* spp. ticks were identified.

Eighteen ticks were found to be positive for spotted fever rickettsial DNA. The DNA of *R. africae* was detected in a *Hyalomma detritum* tick isolated from a wild boar (Table 1), although DNA of *C. Rickettsia barbariae* was found in 7 ticks: 5 *Rh. turanicus* and 2 *Rh. sanguineus*, all of them flagged from the vegetation. Nine ticks were tested positive for *R. massiliae* DNA, including 6 questing *Rh. turanicus* found on vegetation, 1 *Rh. sanguineus* from a dog, and 2 *Haemaphysalis erinacei* from a hedgehog. The DNA of *R. sibirica mongolitimonae* was detected in one *Rh. (B.) annulatus* tick picked from a Mesopotamian fallow deer.

Nucleotide *ompA* sequences of *R. massiliae* (KJ187075-KJ187077), *R. sibirica mongolitimonae* (JF700255), and *C. Rickettsia barbariae* (JF700253) identified in this study were identical to the respective reference sequences deposited for each species in the NCBI GenBank. A 491 base pair (bp) fragment of *ompA* from *R. africae* (JF700254) had a 100%

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TABLE 1
Prevalence of spotted fever group *Rickettsia* DNA in ticks collected from different hosts

<i>Rickettsia</i> species	Tick species tested	Tick found on	No. positive samples	Site of collection
<i>Rickettsia africae</i>	<i>Hyalomma detritum</i>	Wild boar	1	Golan Heights
<i>Candidatus Rickettsia barbariae</i>	<i>Rhipicephalus turanicus</i>	Vegetation	5	Central Israel
	<i>Rhipicephalus sanguineus</i>	Vegetation	2	Central Israel
<i>Rickettsia massiliae</i>	<i>Rhipicephalus turanicus</i>	Vegetation	6	Central Israel
	<i>Rhipicephalus sanguineus</i>	Dog	1	Jerusalem
	<i>Haemaphysalis erinacei</i>	Hedgehog	2	Central Israel
<i>Rickettsia sibirica mongolitimonae</i>	<i>Rhipicephalus (Boophilus) annulatus</i>	Mesopotamian fallow deer	1	Carmel Mountains

sequence similarity with a homologous fragment of *R. africae* (HQ335132) detected in *Hyalomma marginatum* from Qalet El-Nakhl, in the Sinai Peninsula in Egypt.¹³ Both molecular isolates had several nucleotide differences and 99% nucleotide sequence similarity with the reference strain of *R. africae* (CP001612.1). The differences were apparently synonymous substitutions because its predicted protein sequence was the same as that of *R. africae* ESF 2500-1.

DISCUSSION

Molecular techniques are enabling biologists to identify various spotted fever *Rickettsia* species in ticks, and hence provide new evidence for their distribution. The significance of these findings may be important to the attending clinician from the point of the presentation of the disease symptoms and the differentiation from other SFG rickettsial diseases. In addition, the possibility of different SFG rickettsiae being resistant or more sensitive to specific antibiotics may be of life saving importance.¹⁴ Furthermore, specific identification of the rickettsial species allows the tracking and monitoring of potential rickettsial outbreaks.

Rickettsia africae was first isolated by Kelly and others in 1992 from a patient with African tick bite fever and it is now recognized as an important emerging infectious tick borne rickettsial disease in sub-Saharan Africa and in the French West Indies.¹⁵ In this study, we have obtained further evidence of the presence of *R. africae* in Israel found in a *Hyalomma detritum* tick from a wild boar. Recently, *R. africae* has also been detected in *Hyalomma turanicum*, *Hyalomma impeltatum*, *Hyalomma dromedarii* and *Hyalomma excavatum* ticks in Israel.²

Rickettsia africae is commonly vectored by *Amblyomma* spp. ticks in Africa,¹⁶ *Amblyomma variegatum* in the sub-Saharan area, and *Amblyomma hebraeum* in South Africa. The only other region in the world outside of Africa in which *R. africae* has been identified is in the West Indies in *A. variegatum* ticks.¹⁷ It is unclear how or when *R. africae* reached the West Indies but it has been proposed that the rickettsiae may have been present in *A. variegatum* ticks infesting cattle shipped from Senegal during the 18th and 19th centuries.¹⁷ A recent study has unexpectedly detected *R. africae* in *Amblyomma loculosum* ticks collected from humans and birds in New Caledonia.¹⁸

The specificity of *Amblyomma* spp. ticks association with *R. africae* has been further brought into question by the detection of *R. africae* in *Rh. (Boophilus) decoloratus* ticks collected from an oryx in Botswana in 2007.¹⁹ The authors suggested that the presence of *R. africae* in *Rh. (B.) decoloratus* may be due to a blood meal and that the ticks may have just been

passive carriers of the *Rickettsia*. A later publication in a 2010 study in Senegal showed the presence of *R. africae* in *Rh. evertsi evertsi* and in *Rh. (B.) annulatus* (18). Infection rates of the *Rh. evertsi evertsi* and *Rh. (B.) annulatus* were found to be low compared with that of *A. variegatum* with *R. africae*, which may be explained by the lower genus/specificity to *R. africae*.²⁰ All the previously mentioned ticks with the exception of *Rh. (B.) annulatus* are not present in Israel. The presence of *R. africae* in *H. detritum* in Israel is unexpected and novel, however the fact that this *Rickettsia* has been found in a number of species of ixodid ticks and is not specific for only one species strengthens this feasibility. Whether *H. detritum* served as a biological vector cannot be stated with certainty at this stage and requires further study. However, the possibility of a *H. detritum* tick serving as a mechanical vector as a result of a blood meal acquisition seems remote.

The presence of *R. africae* in a different tick species in Israel can only be based on speculation, e.g., global warming,²¹ live-stock movements, and/or migrating birds.²² Israel is located at the junction of three continents and is crossed by a very large number of migrating birds.²³ Studies over the past decade show that about 500 million birds cross Israel's narrow airspace twice every year in the course of their migrations.

To date, infection with *R. africae* has only been made on Israeli travelers to sub-Saharan Africa.^{24,25} Now that the *R. africae* has been found in ticks in Israel, clinicians in Israel should be aware that the diagnosis of the African tick-bite fever may also be made in residents of Israel who have not necessarily traveled to Africa or the West Indies.

Another novel finding in this study was the detection of *R. sibirica mongolitimonae* DNA in an *Rh. (B.) annulatus* tick from a Mesopotamian fallow deer. This is the third occasion that this *Rickettsia*, the cause of "lymphangitis-associated rickettsiosis" have been identified in Israel, with two previous detections made in *Hyalomma* sp. ticks.^{2,3} Our study expanded the associations of *R. sibirica mongolitimonae* to *Rh. (B.) annulatus* ticks. The significance and vector capacity of *Rhipicephalus* spp. ticks for *R. sibirica mongolitimonae* needs further evaluation.

C. Rickettsia barbariae was first identified in *Rh. turanicus* ticks in Portugal and later in Sardinia.²⁶ In this study *C. Rickettsia barbariae* was identified for the first time in *Rh. sanguineus* and *Rh. turanicus* ticks in Israel.

As in previous studies, *R. massiliae* was found in a number of *Rhipicephalus* spp. ticks and on a *H. erinacei* tick, underlining the ubiquity of this *Rickettsia* in Israel.^{2,3}

This study again raises the possibility that, in addition to commonly diagnosed cases of Mediterranean spotted fever caused by *R. conorii*, a number of spotted fever rickettsiosis cases reported in Israel might be caused by other species of

Rickettsia. The pathogenicity of some of these rickettsiae is as yet unclear, however, as with other rickettsiae some could emerge as human pathogens in the future.

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