Am. J. Trop. Med. Hyg., 85(5), 2011, pp. 919–923
doi:10.4269/ajtmh.2011.10-0623

Spotted Fever Group Rickettsiae in Ticks Collected from Wild Animals in Israel

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Abstract. We report molecular evidence for the presence of spotted fever group rickettsiae (SFGR) in ticks collected from roe deer, addax, red foxes, and wild boars in Israel. *Rickettsia aeschlimannii* was detected in *Hyalomma marginatum* and *Hyalomma detritum* while *Rickettsia massiliae* was present in *Rhipicephalus turanicus* ticks. Furthermore, a novel uncultured SFGR was detected in *Haemaphysalis adleri* and *Haemaphysalis parva* ticks from golden jackals. The pathogenicity of the novel SFGR for humans is unknown; however, the presence of multiple SFGR agents should be considered when serological surveillance data from Israel are interpreted because of significant antigenic cross-reactivity among *Rickettsia*. The epidemiology and ecology of SFGR in Israel appear to be more complicated than was previously believed.

INTRODUCTION

Tick-borne spotted fever group (SFG) rickettsioses are caused by obligatory intracellular gram-negative bacteria of the genus *Rickettsia*. In Israel, Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* subsp. *israelensi*s is considered to be the primary cause of spotted fever group rickettsiosis associated with brown dog ticks, *Rhipicephalus sanguineus* Latreille.[1,2]

To gauge the prevalence of SFGR in southern Israel, the hemolymph test showed the presence of rickettsiae in both *Rh. sanguineus* and *Rhipicephalus turanicus* collected from agricultural settlements in Israel.[3] In the Negev region a correlation among the density of domestic animals, their ectoparasites (*Rh. sanguineus*, *Rh. turanicus*, and *Hyalomma* sp. ticks), and the incidence of spotted fever group rickettsiae was demonstrated.[4] Serology was also a sensitive indicator for the presence and magnitude of human and canine exposure to ticks and to SFG rickettsiae (SFGR) based on the prevalence of immunoglobulin G (IgG)-antibodies to *Rickettsia conorii* in two rural villages in Israel.[5] Recently *Rickettsia massiliae* and *Rickettsia sibirica mongolotimonae* were found in questing adult ticks collected from the vegetation in different parts of Israel.[6] Furthermore, the presence of *R. massiliae* DNA sequences was detected in a *Rh. sanguineus* tick picked from the scalp of a pediatric patient in the north of Israel (Keysary A and others, unpublished data).

In Israel ticks such as *Rh. sanguineus* sensu lato, *Rhipicephalus bursa* Canestrini and Fanzago, *Hyalomma marginatum* Koch, and *Haemaphysalis sulcata* Canestrini and Fanzago have been found attached to humans.[7,8] Ixodid ticks, particularly *Rh. sanguineus* and *Rh. turanicus* Pomerantsev, are quite common on domestic and wild animals. *Rhipicephalus* (*Boophilus*) *kohlsi* has been found on the hilly land of the Mediterranean phytogeographic area, mainly on goats and sheep, whereas small numbers of this tick were also found on cattle, mules, horses, and camels.[9] Adults of *H. marginatum* and *Hyalomma detritum* ticks occur on cattle and horses, although their larvae and nymphs can be found on rodents and birds.[10] Previously, *Haemaphysalis adleri* was found on the golden jackal (*Canis aureus*), red fox (*Vulpes vulpes*), jungle cat (*Felis chauss*), and the desert cat (*Felis lybica*), whereas *Haemaphysalis parva* was found on dogs, golden jackal, gray wolf (*Canis lupus*), red fox, and hedgehog (*Erinaceus europeus*).[10,11]

In contrast to studies of domestic animals and humans, there is a dearth of literature on the incidence of SFGR in populations of wild animals and their ticks in Israel. Only a single serological study showed high titers of SFG-rickettisal antibodies was detected in a substantial number of free-ranging jackals (*Canis aureus syriacus*) in Israel.[12]

We report here molecular evidence for the occurrence of two known human pathogenic species of SFGR, *Rickettsia aeschlimannii* in *Hyalomma* ticks, and *Rickettsia massiliae* in *Rh. turanicus* ticks collected from wild animals in Israel. In addition, we report the presence of a novel uncultivated SFGR found in *Haemaphysalis* ticks collected from golden jackals.

MATERIAL AND METHODS

One hundred eighty-one ticks were collected from 6 addax (*Addax nasomaculatus*), 7 red foxes (*V. vulpes*), 5 wild boars (*Sus scrofa*), and 3 golden jackals (*Canis aureus*); and from 4 roe deer (*Capreolus capreolus*) (descendant of European animals that were reintroduced into Israel in the 1980s and 1990s).[13]

Tick collections were performed randomly from animals that had been immobilized for different reasons, live-trapped or found dead. Fallow deer were sampled at Hai Bar Carmel in the north of Israel, addax at Hai Bar Yotvata in the Arava Rift valley in the south of Israel, and the rest from different sites (Table 1).

Ticks were identified to species using standard taxonomic keys[9,11,14–16] and comprised 40 *Rh. sanguineus*, 63 *Rh. turanicus*, 14 *Rhipicephalus* (*Boophilus*) *kohlsi* Hoogstraal and Kaiser, 14 *Hyalomma marginatum* Koch, 15 *Hyalomma dentritum*, 13 *Haemaphysalis adleri* Feldman-Muhsam, and 16 *Haemaphysalis parva* Neumann (Table 1). The six specimens of *Hyalomma* ticks were identified only to the genus.

The ticks were kept in 70% ethanol. DNA was extracted using the QIAamp Minikit (QIAGEN Inc., Valencia, CA), according to the manufacturer's instructions.

Tick extracts were first tested for rickettsial DNA by nested polymerase chain reaction (PCR) to amplify a fragment of 17 kDa protein antigen gene followed by restriction fragment

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

Tick species	Host	Site of collection	Number of PCR-positive ticks/number of ticks tested	Sequence identification (NCBI GenBank accession no.)
Rhipicephalus turanicus	Roe deer	Carmel Mountains (northern Israel)	22/41	*R. massiliae* (GQ856265, GQ856267)
	Red fox	Ein Chemed Even Yehuda (central Israel)	2/17	
	Boar	Granot (northern Israel)	1/5	
Hyalomma marginatum	Roe deer	Carmel Mountains (northern Israel)	10/14	*R. aeschlimannii* (GQ856266, GQ856268)
Hyalomma dentritum	Addax	Yotveta Hai-Bar (southern Israel)	2/15	
Hyalomma sp.	Boar	Ein Yakov (northern Israel)	1/4	
	Red fox	Kfar Shimon (central Israel)	1/2	
Haemaphysalis adleri	Golden jackal	Wadi Ara (central Israel)	1/5	"*Candidatus Rickettsia goldwasserii*" HM136923-HM136926
Haemaphysalis parva	Golden jackal	Wadi Ara (central Israel)	1/8	"*Candidatus Rickettsia goldwasserii*" HM136927-HM136930
Rhipicephalus sanguineus	Roe deer	Carmel Mountains (northern Israel)	0/40	Not applicable
Rhipicephalus (*Boophilus*) *kohlsi*	Roe deer	Carmel Mountains (northern Israel)	0/14	
Haemaphysalis adleri	Red fox	Beth-Shemesh (central Israel)	0/6	
	Boar	Golan Heights (northern Israel)	0/2	
Haemaphysalis parva	Red fox	Beth-Shemesh (central Israel)	0/2	
	Boar	Golan Heights (northern Israel)	0/6	

* PCR = polymerase chain reaction.

length polymorphism analysis as described previously.[17] Species identification of SFG *Rickettsia* was done by sequencing of 70–602 nucleotide fragments of the outer membrane protein A (OmpA) and 17 kDa protein gene fragments as described previously.[18] Multiple locus sequence analysis included amplification and sequencing fragments of *glt*A, *omp*A, *omp*B, and *sca*4 as described previously.[18–20] New sequences generated during this study were submitted to NCBI GenBank under the following accession nos.: *R. aeschlimannii* - GQ856266 and GQ856268, *R. massiliae* - GQ856265 and GQ856267, and uncultured SFGR - HM136923-HM136930. Phylogenetic analysis was conducted using MEGA4.[21]

RESULTS

DNA was extracted from 181 ticks collected from 25 wild animals of five species: roe deer, addax, red foxes, golden jackals, and wild boars (Table 1). DNA of SFGR was detected in 41 ticks (22.7% prevalence for the entire study).

DNA of *R. massilliae* was detected in 25 of 63 *Rh. turanicus* ticks, collected from roe deer, foxes, and boars. Nucleotide sequences were identical among all *omp*A amplicons obtained from *Rh. turanicus* and had 99% nucleotide identity to the homologous *omp*A fragment of both *R. massiliae* Mtu5 and Bar29 and 100% similarity to Mtu1 strain.

No *omp*A sequence differences were observed in DNA from *R. aeschlimannii* detected in 14 *Hyalomma* ticks out of 35 collected from roe deer, addax, boars and foxes. DNA of a novel SFGR was found in two *Haemaphysalis* species ticks: *H. adleri* (in 1 of 5 ticks) and *H. parva* (in 1 of 8 ticks) from golden jackals (Table 1). Homologous rickettsial fragments of *glt*A, *omp*A, *omp*B, and *sca*4 amplified from these two different ticks were found to be identical. The 381 bp *glt*A fragment sequenced had 99% sequence similarity and at least 1 to 3 unique single-nucleotide polymorphisms (SNP) compared with *glt*A fragment of *Rickettsia honei*, *Rickettsia africae*, *Rickettsia slovaca*, and *Rickettsia japonica*. The 575 bp *omp*A fragment had < 96% sequence similarity to *omp*A of *R. honei* and *R. slovaca*, *R. africae* and *Rickettsia sibirica mongolotimonae*, corresponding to 18–21 SNP. The 1,765 bp *sca*4 fragment had < 97% sequence similarity to the homologous fragments of *R. slovaca* and other SFGR encompassing multiple SNPs and several unique insertion/deletion (INDEL). The 4,899 bp *omp*B fragment had ≤ 97% sequence similarity to the nearest rickettsial relative. Phylogenetic analysis of the four concatenated gene fragments, *glt*A-*omp*A-*sca*4-*omp*B indicated that the nucleotide sequences are those of a SFGR belonging to a novel phylogenetic lineage that appears to be most related to "*Candidatus* Rickettsia siciliensis" that was recently found in *Rh. turanicus* (Figure 1).[22] "*Candidatus* Rickettsia siciliensis" and the SFGR from *H. adleri* and *H. parva* ticks share 99%, 95%, 98%, and 96% nucleotide sequence similarity in their homologous fragments of *glt*A (HM014438), *omp*A (HM014439), *sca*4 (HM014440), and *omp*B (HM014441), respectively.

Rickettsial DNA was not detected in *Rh. sanguineus* and *Rh.* (*B.*) *kohlsi* collected on roe deer or *H. adleri* from boar and red fox (Table 1).

DISCUSSION

We detected *R. aeschlimannii* in *Hyalomma* sp. ticks and *R. massiliae* in *Rh. turanicus* ticks collected from wild animals and captive bred wildlife in Israel (Table 1). We also characterized a previously unknown SFGR in *H. adleri* and *H. parva* from jackals.

Rickettsia aeschlimannii was first described from *H. marginatum marginatum* ticks from Morocco in 1997[23] and was subsequently detected in 2002 in a patient returning from Morocco.[24] Subsequently, the presence of *R. aeschlimannii* has been demonstrated in *H. marginatum marginatum* ticks with a distribution from Portugal and northern Spain to Kazakhstan and from Mediterranean countries to South Africa.[25,26] The prevalence of *R. aeschlimannii* in *H. marginatum* ticks tested ranges from 1.8% to 64% in different studies.[23,27–29] *Rickettsia aeschlimannii* has also been detected in *Hyalomma aegyptum* (L.) in Algeria,[30] *Haemaphysalis inermis* Birula in Spain,[27] *Hyalomma marginatum rufipes* Koch in Egypt, Ethiopia, and Chad,[28,31] *Hyalomma anatolicum excavatum* Koch from the Greek Island of Cephalonia,[32] and *Hyalomma dromedarii* Koch and

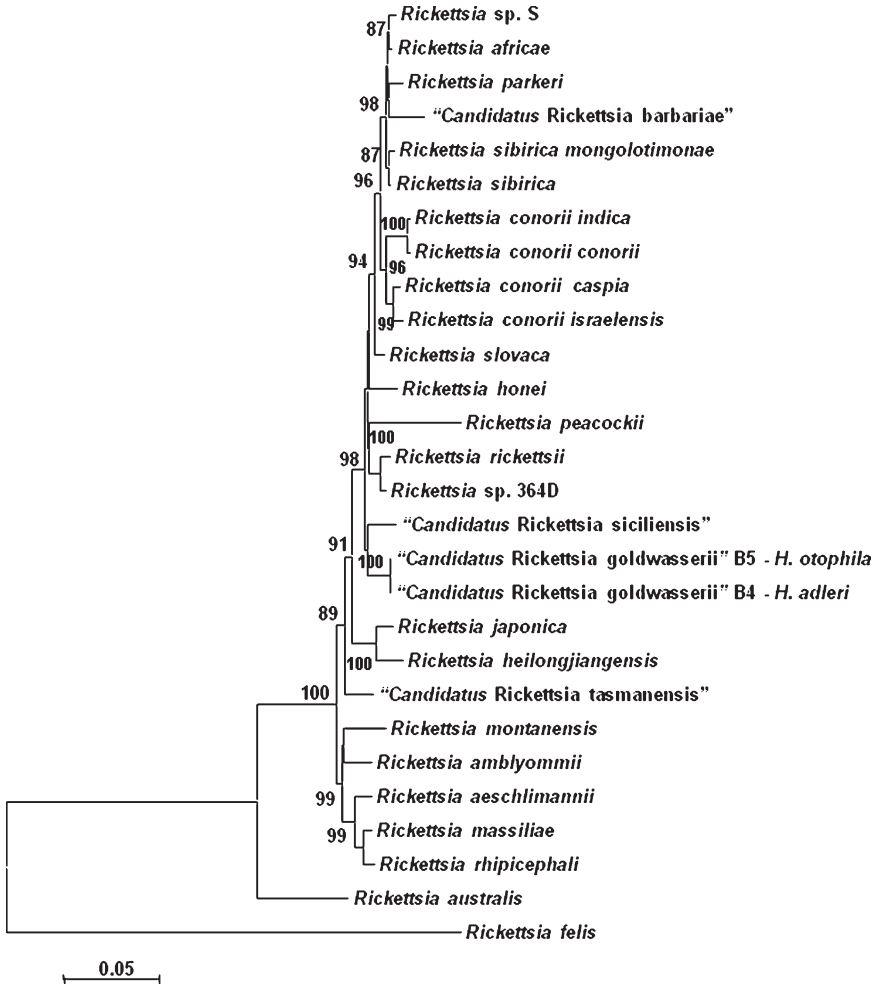

FIGURE 1. Phylogenetic position of "*Candidatus Rickettsia goldwasserii*." Phylogenetic position was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.78594827 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches; only bootstrap values of ≥ 80 are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method as base substitutions per site. All positions containing gaps, missing data and primer sequences were eliminated from the dataset. Fragments of the four genes were concatenated (*glt*A-*omp*A-*sca*4-*omp*B), and a total of 2,028 positions were analyzed. Phylogenetic analyses were conducted with MEGA4.[21]

Hyalomma impeltatum Schulze and Schlottke from Egypt.[31] Furthermore, five other human biting tick species including *Haemaphysalis punctata* Canestrini and Fanzago, *Ixodes ricinus* (L.), *Rh. bursa*, and *Rh. sanguineus* collected from Spanish patients were shown to contain DNA of *R. aeschlimannii.*[33] The association of *Rhipicephalus* complex ticks with *R. aeschlimannii* is probably not surprising, because it was also detected in *Rhipicephalus apendiculatus* Neumann ticks collected from a patient suffering from *R. aeschlimannii* infection.[34]

Rickettsia massiliae has been detected in *Rh. sanguineus*, *Rhipicephalus sulcatus* Neumann, *Rhipicephalus lunulatus* Neumann, *Rhipicephalus muhsamae* Morel and Vassiliades, *Rhipicephalus senegalensis* Koch, *Rh. bursa*, and *Rh. turanicus*.[23,28,35–37] *Rickettsia massiliae* has been detected in *Rhipicephalus* ticks in Europe, Africa, and in South and North America.[37–39] There are several confirmed clinical cases caused by *R. massiliae* reported in the peer-reviewed literature.[37–39] Furthermore, it is believed that *R. massiliae* is responsible for cases of SFG rickettsioses resistant to rifampin in Catalonia, Spain,[39] which corresponds closely to the observation that *R. massiliae* is naturally resistant to this antibiotic.[40]

The SFGR detected in *H. adleri* and *H. parva* has unique genetic characteristics that meet the minimum current requirement for identification as a new species based on the proposed molecular similarity criteria: ≤ 99.9%, ≤ 98.8%, ≤ 99.2%, and ≤ 99.3% for the *glt*A, *ompA* and *ompB*, and *sca*4, respectively, to its closest SFGR relative.[27,41] We cannot propose a formal species description because only two specimens from different tick species were analyzed and a rickettsial isolate was not established to complete its characterization. However, we can assign *Candidatus* status to this yet uncultivated SFGR *Rickettsia* and name it "*Candidatus Rickettsia goldwasserii*" in recognition of Dr. Robert A. Goldwasser for his work on rickettsiae and rickettsial diseases in Israel and his important contributions to the development of indirect fluorescent antibody assays for rickettsiae.[42,43] Further detailed study will be necessary to establish the prevalence and distribution of this SFGR in *Haemaphysalis* ticks from Israel, its primary vector

and reservoir, and its ability to cause human and animal rickettsioses.

In Israel, ixodid ticks, which have been found on humans include 13 various species of *Hyalomma*, the sheep tick, *Rh. bursa* and *H. sulcata* as documented by Feldman-Muhsam.[8] Cwilich and Hadani found *H. excavatum, H. detritum, H. marginatum, Rh. sanguineus, Rh. turanicus*, and *Rh. bursa* on humans.[7] To the best of our knowledge, there is no published document showing that *H. parva* and *H. adleri* infest humans.

Our results provide evidence for the presence of *R. aeschlimannii* and confirm the evidence for the presence of *R. massiliae* in Israel.[6] Further surveillance will be needed to characterize tick and animal reservoir for each SFGR reported.

Serological diagnosis of spotted fever infections cannot distinguish between those caused by *R. massiliae*, *R. aeschlimannii*, and *R. conorii* or potentially other SFGR because of the strong cross-reaction among the spotted fever group rickettsiae. Definitive diagnosis of the specific etiological SFGR agent causing rickettsioses in Israel requires molecular techniques conducted on whole blood and skin biopsy samples collected during the acute stage of illness and before antibiotic administration.

Received November 2, 2010. Accepted for publication June 6, 2011.

Acknowledgments: We thank Arianna Salazar for laboratory assistance, rangers of the Israel Nature and Parks Authority for field assistance, and Gregory A. Dasch for reviewing the manuscript and helpful suggestions.

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REFERENCES

1. Aharonowitz G, Koton S, Segal S, Anis E, Green MS, 1999. Epidemiological characteristics of spotted fever in Israel over 26 years. *Clin Infect Dis 29:* 1321–1322.
2. Mumcuoglu KY, Keysary A, Gilead L, 2002. Mediterranean spotted fever in Israel: a tick-borne disease. *Isr Med Assoc J 4:* 44–49.
3. Guberman D, Mumcuoglu KY, Keysary A, Ioffe-Uspensky I, Miller J, Galun R, 1996. Prevalence of spotted fever group rickettsiae in ticks from southern Israel. *J Med Entomol 33:* 979–982.
4. Mumcuoglu KY, Ioffe-Uspensky I, Alkrinawi S, Sarov B, Manor E, Galun R, 2001. Prevalence of vectors of the spotted fever group Rickettsiae and murine typhus in a Bedouin town in Israel. *J Med Entomol 38:* 458–461.
5. Harrus S, Lior Y, Ephros M, Grisaru-Soen G, Keysary A, Strenger C, Jongejan F, Waner T, Baneth G, 2007. *Rickettsia conorii* in humans and dogs: a seroepidemiologic survey of two rural villages in Israel. *Am J Trop Med Hyg 77:* 133–135.
6. Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, Baneth G, 2011. Molecular detection of *Rickettsia massiliae, Rickettsia sibirica mongolitimonae* and *Rickettsia conorii israelensis* in ticks from Israel. *Clin Microbiol Infect 17:* 176–180.
7. Cwilich R, Hadani A, 1969. A note on some cases of human infestation with "hard" ticks (Ixodoidea, Ixodidae). *Refu Vet 26:* 79–81.
8. Feldman-Muhsam B, 1986. Ixodid tick attacks on man in Israel: medical implications. *Isr J Med Sci 22:* 19–23.
9. Feldman-Muhsam B, Shechter R, 1970. Some notes on the genus *Boophilus* (Ixodidae), with special reference to species found in Israel. *J Med Entomol 7:* 677–686.
10. Theodor O, Costa M, 1967. Part 1. Ectoparasites. *A Survey of the Parasites of Wild Mammals and Birds in Israel*. Jerusalem: The Israel Academy of Sciences and Humanities, 117.
11. Feldman-Muhsam B, 1951. A note on East Mediterranean species of the *Haemophysalis*. *Bull Res Counc Isr 1:* 96–107.
12. Waner T, Baneth G, Strenger C, Keysary A, King R, Harrus S, 1999. Antibodies reactive with *Ehrlichia canis, Ehrlichia phagocytophila* genogroup antigens and the spotted fever group rickettsial antigens, in free-ranging jackals (*Canis aureus syriacus*) from Israel. *Vet Parasitol 82:* 121–128.
13. Wallach AD, Shanas U, Mumcuoglu KY, Inbar M, 2008. Ectoparasites on reintroduced roe deer *Capreolus capreolus* in Israel. *J Wildl Dis 44:* 693–696.
14. Pegram R, Clifford C, Walker J, Keirans J, 1987. Clarification of the *Rhipicephalus sanguineus* group (Acari, Ixodoidea, Ixodidae). I. *R. sulcatus* Neumann, 1908 and *R. turanicus* Pomerantsev, 1936. *Syst Parasitol 10:* 3–26.
15. Walker J, Keirans J, Horak I, 2000. The genus *Rhipicephalus* (Acari, Ixoidae). *A Guide to the Brown Ticks of the World*. Cambridge: Cambridge University Press.
16. Feldman-Muhsam B, 1954. Revision of the genus *Hyalomma*. *Bull Res Counc Isr 64:* 150–170.
17. Leitner M, Yitzhaki S, Rzotkiewicz S, Keysary A, 2002. Polymerase chain reaction-based diagnosis of Mediterranean spotted fever in serum and tissue samples. *Am J Trop Med Hyg 67:* 166–169.
18. Eremeeva ME, Bosserman EA, Demma LJ, Zambrano ML, Blau DM, Dasch GA, 2006. Isolation and identification of *Rickettsia massiliae* from *Rhipicephalus sanguineus* ticks collected in Arizona. *Appl Environ Microbiol 72:* 5569–5577.
19. Sekeyova Z, Roux V, Raoult D, 2001. Phylogeny of *Rickettsia* spp. inferred by comparing sequences of 'gene D', which encodes an intracytoplasmic protein. *Int J Syst Evol Microbiol 51:* 1353–1360.
20. Roux V, Raoult D, 2000. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*omp*B). *Int J Syst Evol Microbiol 50:* 1449–1455.
21. Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol 24:* 1596–1599.
22. Eremeeva ME, Stromdahl EY, 2011. Short report: new spotted fever group *Rickettsia* in a *Rhipicephalus turanicus* tick removed from a child in eastern Sicily, Italy. *Am J Trop Med Hyg 84:* 99–101.
23. 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.
24. Raoult D, Fournier PE, Abboud P, Caron F, 2002. First documented human *Rickettsia aeschlimannii* infection. *Emerg Infect Dis 8:* 748–749.
25. Parola P, 2006. Rickettsioses in sub-Saharan Africa. *Ann N Y Acad Sci 1078:* 42–47.
26. Shpynov S, Fournier PE, Rudakov N, Tankibaev M, Tarasevich I, Raoult D, 2004. Detection of a *Rickettsia* closely related to *Rickettsia aeschlimannii*, *"Rickettsia heilongjiangensis," Rickettsia* sp. strain RpA4, and *Ehrlichia muris* in ticks collected in Russia and Kazakhstan. *J Clin Microbiol 42:* 2221–2223.
27. Portillo A, Santibanez P, Santibanez S, Perez-Martinez L, Oteo JA, 2008. Detection of *Rickettsia* spp. in *Haemaphysalis* ticks collected in La Rioja, Spain. *Vector Borne Zoonotic Dis 8:* 653–658.
28. Mura A, Socolovschi C, Ginesta J, Lafrance B, Magnan S, Rolain JM, Davoust B, Raoult D, Parola P, 2008. Molecular detection of spotted fever group rickettsiae in ticks from Ethiopia and Chad. *Trans R Soc Trop Med Hyg 102:* 945–949.

29. Fernandez-Soto P, Diaz Martin V, Perez-Sanchez R, Encinas-Grandes A, 2009. Increased prevalence of *Rickettsia aeschlimannii* in Castilla y Leon, Spain. *Eur J Clin Microbiol Infect Dis 28:* 693–695.
30. Bitam I, Parola P, Matsumoto K, Rolain JM, Baziz B, Boubidi SC, Harrat Z, Belkaid M, Raoult D, 2006. First molecular detection of *R. conorii*, *R. aeschlimannii*, and *R. massiliae* in ticks from Algeria. *Ann N Y Acad Sci 1078:* 368–372.
31. Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy IM, Moriarity JR, Dasch GA, 2006. Rickettsial agents in Egyptian ticks collected from domestic animals. *Exp Appl Acarol 40:* 67–81.
32. Psaroulaki A, Ragiadakou D, Kouris G, Papadopoulos B, Chaniotis B, Tselentis Y, 2006. Ticks, tick-borne rickettsiae, and *Coxiella burnetii* in the Greek Island of Cephalonia. *Ann N Y Acad Sci 1078:* 389–399.
33. Fernandez-Soto P, Encinas-Grandes A, Perez-Sanchez R, 2003. *Rickettsia aeschlimannii* in Spain: molecular evidence in *Hyalomma marginatum* and five other tick species that feed on humans. *Emerg Infect Dis 9:* 889–890.
34. Pretorius AM, Birtles RJ, 2002. *Rickettsia aeschlimannii*: a new pathogenic spotted fever group *Rickettsia*, South Africa. *Emerg Infect Dis 8:* 874.
35. Brouqui P, Parola P, Fournier PE, Raoult D, 2007. Spotted fever rickettsioses in southern and eastern Europe. *FEMS Immunol Med Microbiol 49:* 2–12.
36. Letaief A, 2006. Epidemiology of rickettsioses in North Africa. *Ann N Y Acad Sci 1078:* 34–41.
37. Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, Labauge P, Raoult D, 2008. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis 2:* e338.
38. Garcia-Garcia JC, Portillo A, Nunez MJ, Santibanez S, Castro B, Oteo JA, 2010. A patient from Argentina infected with *Rickettsia massiliae*. *Am J Trop Med Hyg 82:* 691–692.
39. Vitale G, Mansuelo S, Rolain JM, Raoult D, 2006. *Rickettsia massiliae* human isolation. *Emerg Infect Dis 12:* 174–175.
40. Rolain JM, Maurin M, Vestris G, Raoult D, 1998. *In vitro* susceptibilities of 27 rickettsiae to 13 antimicrobials. *Antimicrob Agents Chemother 42:* 1537–1541.
41. Raoult D, Fournier PE, Eremeeva M, Graves S, Kelly PJ, Oteo JA, Sekeyova Z, Tamura A, Tarasevich I, Zhang L, 2005. Naming of Rickettsiae and rickettsial diseases. *Ann N Y Acad Sci 1063:* 1–12.
42. Murray RG, Stackebrandt E, 1995. Taxonomic note: implementation of the provisional status *Candidatus* for incompletely described procaryotes. *Int J Syst Bacteriol 45:* 186–187.
43. Goldwasser RA, Steiman Y, Klingberg W, Swartz TA, Klingberg MA, 1974. The isolation of strains of rickettsiae of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. *Scand J Infect Dis 6:* 53–62.