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Rickettsia Species in Ticks Removed from Humans in Istanbul, Turkey

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Abstract

A total of 167 ticks collected from humans in Istanbul (Turkey) in 2006 were screened for *Rickettsia* species, and nested PCRs targeting *gltA* and *ompA* rickettsial fragment genes were carried out. *Rickettsia monacensis* (51), *R. aeschlimannii* (8), *R. conorii* subsp. *conorii* (3), *R. helvetica* (2), *R. raoultii* (1), *R. africae* (1), *R. felis* (1), and other *Rickettsia* spp. (2), were detected. To our knowledge, these *Rickettsia* species (except *R. conorii*) had never been reported in ticks removed from humans in Turkey. The presence of *R. africae* also had not been previously described, either in *Hyalomma* ticks or in any European tick species. In addition, *R. aeschlimannii* and *R. felis* had not been found associated with *Rhipicephalus bursa* specimens. The presence of human pathogenic *Rickettsia* in ticks removed from humans provides information about the risk of tick-borne rickettsioses in Turkey.

Key Words: *Rickettsia* species—Turkey—Ticks from humans.

Introduction

Rickettsia species are gram-negative intracellular bacteria that cause severe human disease worldwide. Human rickettsioses include the spotted fever group (SFG) diseases, which are mainly transmitted by hard ticks (Hechemy et al. 2006). In Turkey, ticks that bite humans belong to the genera *Ixodes*, *Hyalomma*, *Dermacentor*, *Rhipicephalus,* and *Haemaphysalis* (Vatansever et al. 2008; Bursali et al. 2010, 2011; Karaer et al. 2011). Species of these genera have been recognized as vectors and reservoirs of *Rickettsia* spp. (Parola et al. 2005; Hechemy et al. 2006;). However, to date, only Mediterranean spotted fever (MSF), caused by *Rickettsia conorii*, has been reported from humans in Turkey (Kuloglu et al. 2004). Autochthonous SFG rickettsioses have been described in Europe: MSF, lymphangitis-associated rickettsioses (LAR), *Dermacentor*-borne necrosis erythema lymphadenopathy (DEBONEL)/tick-borne lymphadenopathy (TIBOLA), *Rickettsia massiliae* infection, *Rickettsia helvetica* infection, and *Rickettsia monacensis* infection (Oteo and Portillo 2012). In addition, African tick-bite fever (ATBF) and *Rickettsia aeschlimannii* infection have been considered imported rickettsioses (from Africa). The location of Turkey, an Eurasian country close to Africa (Fig. 1), as well as its abundance and diversity of ticks (Aydin and Bakirci 2007; Vatansever et al. 2008), makes this country a suitable place for the circulation of tick-borne pathogens. The aim of this study was to investigate the presence of *Rickettsia* spp. in ticks that bite humans in the area of Istanbul.

Materials and Methods

In 2006, a total of 1054 ticks were removed from humans in more than 25 hospitals in Istanbul (Turkey), as part of a project supported by the Ministry of Health, Istanbul Branch (Vatansever et al. 2008). Among them, 167 were randomly chosen for this study. The ticks were classified by taxonomic keys (Walker et al. 2003; Apanaskevich 2003; Estrada-Peña et al. 2004). In addition, specimens whose phenotypic classification was not conclusive were also classified by genotypic methods (PCR of the 16S rDNA gene; Black and Piesman 1994). DNA was individually extracted with the Qiagen Dneasy blood/tissue kit (Qiagen, Hilden, Germany), according to the manufacturer's tissue protocol instructions. DNA extracts were used as templates for PCR assays targeting the 16S rDNA gene for tick classification as cited above, as well as the rickettsial citrate synthase (*gltA*), and 190-kDa protein antigen (*ompA*) genes. PCR primer pairs, size of the amplicons (bp), and annealing temperatures of the assays, are shown in Table 1. Two negative controls, one of them with template DNA but without primers, and the other with primers and containing water instead of template DNA, as well as positive

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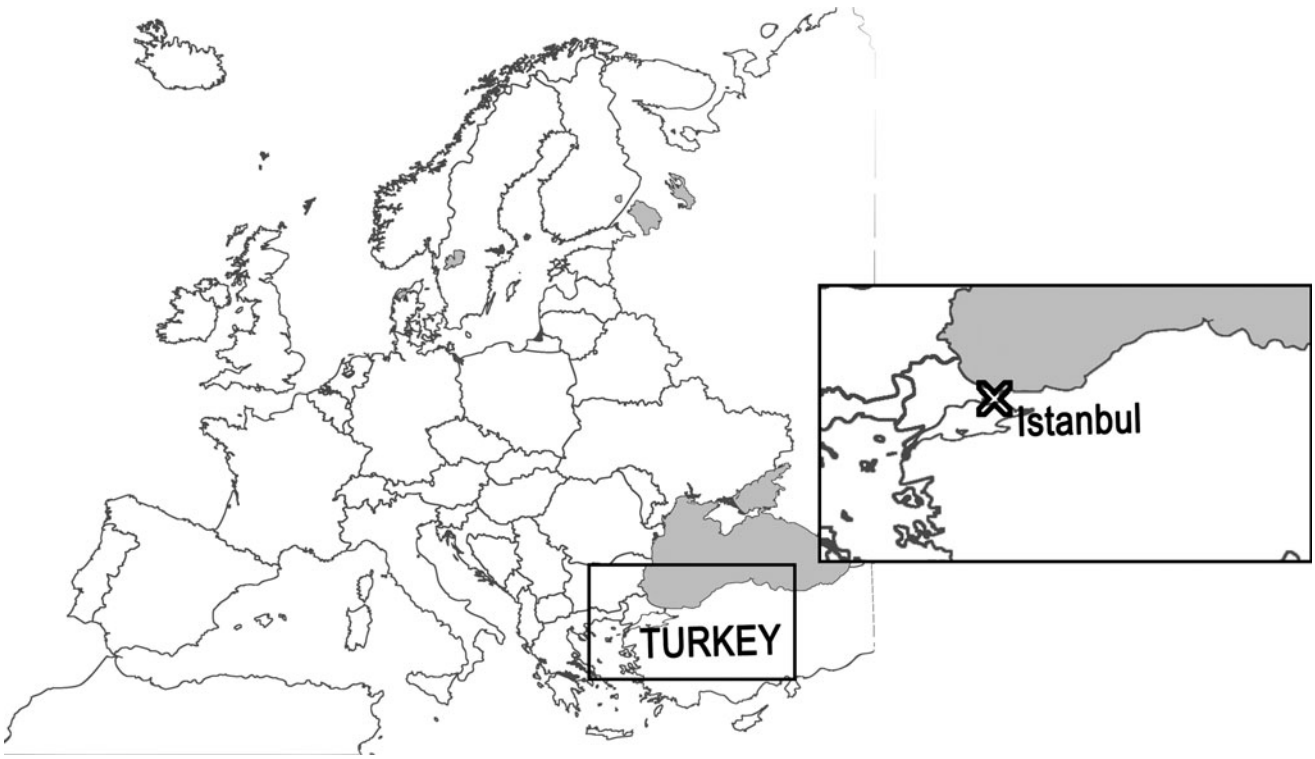

FIG. 1. Map of Europe showing the location of Istanbul.

controls (a tick extract and *Rickettsia slovaca*, respectively) were included in all PCR assays. *Rickettsia* spp. were screened by *gltA* PCR. Positive samples were further tested by *ompA* PCR, and the obtained amplicons were sequenced for the differentiation of the SFG rickettsia species. However, *gltA* amplicons were only sequenced from samples that yielded negative PCR results for *ompA*. Nucleotide sequences were compared with those available in GenBank using the National Center for Biotechnology Information (NCBI; Bethesda, MD) Basic Local Alignment Sequence Tool (BLAST) search engine (http://blast.ncbi.nlm.nih.gov/blast.cgi).

Results

Studied ticks belonged to the following species: *Ixodes ricinus* ($n=130$), *Rhipicephalus sanguineus* group ($n=19$), *Hyalomma aegyptium* ($n=11$), *Hyalomma* spp. ($n=4$), *Hyalomma marginatum* ($n=2$), and *Dermacentor marginatus* ($n=1$). Sixty-nine out of 167 samples (41.3%) tested positive for the presence of *Rickettsia* spp. Table 2 shows details about the *Rickettsia* species detected and *Rickettsia*-infected tick species, as well as the highest percentages of identity with a validly-published *Rickettsia* species. According to *ompA* nucleotide sequences, *R. monacensis,* which was detected in 49 *I. ricinus* (70%) ticks, was the most prevalent genospecies. On the one hand, *R. aeschlimannii* was found in 8 tick specimens (12%): 5 *H. aegyptium*, 2 *H. marginatum,* and 1 *Rhipicephalus bursa*. On the other hand, *R. conorii* subsp. *conorii* was detected in 3 *R. bursa* (4%) specimens, whereas *R. africae*, *R. felis,* and *R. raoultii* appeared in 1 *H. aegyptium*, 1 *R. bursa,* and 1 *D. marginatus,* specimens, respectively. According to *gltA* nucleotide sequences (from samples with negative *ompA* PCR

Table 1. PCR Primer Pairs Used in This Study

	Gene target	*Primer name*	*Primer sequence 5′→3′*	*Amplified fragment (bp)*	*Annealing temperature (°C)*	*Reference*
Tick species	16S rRNA	16S+1	CTGCTCAATGATTTTTTAAATTGCTGTGG	456	48	Black and Piesman 1994
		16S–1	CCGGTCTGAACTCAGATCAAGT		54	
Rickettsia spp.	*ompA* (semi-nested)	Rr190.70p	ATGGCGAATATTTCTCCAAAA	631	46	Regnery et al. 1991; Roux et al. 1996
		Rr190.701n	GTTCCGTTAATGGCAGCATCT			
		Rr190.70p	ATGGCGAATATTTCTCCAAAA	532	48	
		Rr190.602n	AGTGCAGCATTCGCTCCCCCT			
	gltA central region (nested)	RpCS.877p	GGGGGCCTGCTCACGGCGG	381	48	Regnery et al. 1991; Choi et al. 2005
		RpCS1258n	ATTGCAAAAAGTACAGTGAACA			
		RpCS.896p	GGCTAATGAAGCAGTGATAA	337	54	
		RpCS.1233n	GCGACGGTATACCCATAGC			

Table 2. Genospecies of *Rickettsia* Species Detected in Ticks by Nucleotide Sequence Analysis of *ompA* or *gltA* PCR Products ($n=69$)

Rickettsia *species (number)*	*Tick species (number of each stage)*	*Sequenced amplicon*	*% identity*	*GenBank ID*
R. monacensis (51)	*Ixodes ricinus* (13N-35F-1M)	*ompA*	99.2–100	AF201329
	I. ricinus (2F)	*gltA*	100	AY048817
R. aeschlimannii (8)	*Hyalomma aegyptium* (4N-1M)	*ompA*	98.3–100	U43800
	Hyalomma marginatum (1N-1F)	*ompA*	99.8	U43800
	Rhipicephalus bursa (1M)	*ompA*	98.3	U43800
R. conorii subsp. *conorii* (3)	*R. bursa* (2F-1M)	*ompA*	100	U45244
R. africae (1)	*H. aegyptium* (1N)	*ompA*	99.3	U43790
R. felis (1)	*R. bursa* (1M)	*ompA*	100	AF210694
R. raoultii (1)	*Dermacentor marginatus* (1N)	*ompA*	99.4	DQ365801
R. helvetica (2)	*Ixodes ricinus* (2F)	*gltA*	100	U59723
Rickettsia spp. (2)	*Rhipicephalus sanguineus* (1M)	*gltA*	99.7	[a]
	R. bursa (1N)	*gltA*	99.7	[a]

[a]Same identity with more than one validly published *Rickettsia* species.
The GenBank accession numbers as well as the highest percentages of identity with *Rickettsia* species are also included.
N, nymph; F, female; M, male.

results), *R. helvetica* ($n=2$; 3%), and *R. monacensis* ($n=2$; 3%), were detected in 4 *I. ricinus* ticks. In addition, 2 specimens of the *Rhipicephalus* genus harbored unidentified *Rickettsia* spp. that showed *gltA* sequences with the same percentage of identity with more than one validly-published rickettsia. These sequences shared 99.7% identity with *R. raoultii* (DQ365804), *R. aeschlimannii* (U59722), *Rickettsia japonica* (U59724), or *Rickettsia heilongjiangensis* (AF178034), among other rickettsia species. Unfortunately, analysis of alternative genes to identify rickettsial species could not be performed, since all DNA was used to investigate other tick-borne bacteria (data not shown).

Discussion

To date, *R. conorii* was the unique confirmed tick-borne rickettsia species known in Turkey (Kuloglu et al. 2004). Thus cases of MSF have been reported from this country, and *R. conorii* has been amplified in ticks by PCR (Kuloglu et al. 2004). Our study confirms this fact, as well as the circulation of other rickettsias different from *R. conorii* in ticks removed from humans in Istanbul. We report here the presence of these pathogenic tick-borne bacteria: *R. conorii* subsp. *conorii*, *R. monacensis*, *R. aeschlimannii*, *R. helvetica*, *R. raoultii*, *R. africae,* and *R. felis*.

In this study, we have found *R. conorii* subsp. *conorii* in *R. bursa* specimens attached to humans. This tick species belongs to the same group as *R. sanguineus*, which is the current vector of MSF. These tick species can be easily confused, since their morphological differentiation at some stages is still unclear. The detection of *R. conorii* subsp. *conorii* in *R. bursa* corroborates data from a previous study with ticks collected on livestock in Turkey (Christova et al. 2003). We think that *R. bursa* could also be a vector of *R. conorii,* since we have observed *R. bursa* attached to patients who subsequently developed MSF (Oteo and Portillo 2012). Furthermore, our data have shown the presence of *R. helvetica* and *R. monacensis* in *I. ricinus* ticks. These rickettsia species, mainly transmitted by *I. ricinus*, have been associated with MSF-like diseases previously reported in Europe (Nilsson et al. 1999; Fournier et al. 2000; Jado et al. 2007). In addition, *I. ricinus* is the most prevalent tick species attached to humans in Istanbul analyzed in our study (Vatansever et al. 2008). According to our data, *R. raoultii* has been amplified in *D. marginatus*. This rickettsia species had previously been detected in *Dermacentor reticulatus* and *D. marginatus* that bit humans who developed *R. slovaca*-like infection or TIBOLA/DEBONEL symptoms (Mediannikov et al. 2008). Moreover, we have found *R. aeschlimannii* in *H. aegyptium* and *H. marginatum*, as previously reported, as well as in one *R. bursa* specimen. To our knowledge, this is the first detection of *R. aeschlimannii* in *R. bursa*, although this rickettsia had been amplified in *Rhipicephalus* spp. (Bitam et al. 2009; Mediannikov et al. 2010). Human infection by *R. aeschlimannii* was first reported in Africa (Raoult et al. 2002), and it has been associated with European ticks, although there are no recognized endemic cases of the disease (Oteo et al. 2005; Oteo and Portillo 2012).

Our data have revealed the presence of *R. felis* in *R. bursa*. Molecular evidence of *R. felis* in ticks has been previously reported (Reif et al. 2009), but not in *R. bursa* or in ticks collected in Europe. Nevertheless, fleas are the only known vector of this potential tick-borne pathogen.

R. africae is responsible for ATBF, an infection that has been reported in Africa, and as an imported disease in Europe (Jensenius et al. 2003; Oteo et al. 2004). Until now, *R. africae* had been associated with *Amblyomma* and *Rhipicephalus* species (Kelly et al. 1996; Portillo et al. 2007; Mediannikov et al. 2010). In our study, we found *R. africae* in *H. aegyptium*. This tick species is not a known vector of *R. africae*, but our results demonstrate the circulation of this rickettsial pathogen in the studied environment.

All rickettsia species found in this study are associated with human disease. The presence of several tick-borne pathogens, their vectors, and reservoirs, as well as the evidence of human tick bites, suggest that some of these tick-borne infections are circulating in Turkey and may be misdiagnosed. This information should be taken into account for the correct diagnosis and treatment of tick-borne infections in Turkey.

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Author Disclosure Statement

No competing financial interests exist.

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