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Short communication

Spotted fever group rickettsiae in ticks in Turkey

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ABSTRACT

One hundred twenty-six ticks belonging to 12 tick species were collected from humans, domestic and wild animals, and from the ground as unfed (questing ticks) from distinct localities in Turkey in 2011. Ticks were individually tested by polymerase chain reaction (PCR) for *Rickettsia* spp., amplifying citrate synthase (*gltA*), and outer membrane protein (*ompA*) genes. Twenty-five ticks (19.8%) were found to be infected with *Rickettsia* species. Five SFG rickettsiae were identified, including 4 pathogens: *Ri. aeschlimannii* in *Hyalomma marginatum*, *Hy. aegyptium*, *Hyalomma* sp. (nymph), and *Rhipicephalus turanicus*; *Ri. africae* in *Hy. excavatum*, *Hy. aegyptium*, and *Hyalomma* sp. (nymph); *Ri. slovaca* and *Ri. raoultii* in *Dermacentor marginatus*; and one species with unknown pathogenicity, *Ri. hoogstraalii*, in *Haemaphysalis parva*. *Rickettsia slovaca* and *Ri. hoogstraalii* were reported for the first time from Turkey. In addition, *Ri. hoogstraalii* and *Ri. africae* were detected for the first time in *Ha. parva* and *Hy. excavatum* ticks, respectively.

Introduction

Spotted fever group (SFG) rickettsioses are caused by obligate intracellular bacteria belonging to the genus *Rickettsia* within the family Rickettsiaceae in the order Rickettsiales (Raoult and Roux, 1997). The genus *Rickettsia* has been divided into 3 groups: the 'spotted fever group' (SFG), the 'typhus group' (TG), and the 'scrub typhus group' (STG). It has been known so far that 31 rickettsial species were recognized, and many more species are to be identified (Merhej and Raoult, 2011). These bacteria transmitted by arthropods, mainly ticks, may cause disease in vertebrate hosts, such as humans, domestic animals, birds, and wildlife (Parola et al., 2005). Some of ticks can transmit the rickettsiae both transstadially and transovarially; therefore, ticks are known to be the main reservoirs and vectors of SFG rickettsiae in nature (Socolovschi et al., 2009). This infection can cause spotted fever, and the clinical signs include fever, headache, rash, muscle pain, local lymphadenopathy, and sometimes a characteristic eschar (tache noire) at the side of tick bite in humans (Raoult and Roux, 1997; Parola et al., 2005).

In Turkey, ixodid ticks belong to the genera *Hyalomma*, *Dermacentor*, *Rhipicephalus*, *Haemaphysalis*, and *Ixodes*; argasid ticks belong to the genera *Argas*, *Ornithodoros* and *Otobius*. These ticks often take blood from animals and humans (Estrada-Peña et al., 2004; Aydin and Bakirci, 2007; Karaer et al., 2011). Data regarding occurrence of rickettsiae in ticks and in humans are vague in Turkey. *Ri. monacensis*, *Ri. helvetica*, *Ri. aeschlimannii*, *Ri. conorii* subsp. *conorii*, *Ri. africae*, *Ri. raoultii*, and *Ri. felis* were detected in ticks (Christova et al., 2003; Gargili et al., 2012), whilst *Ri. conorii* was detected in patients in Turkey (Kuloglu et al., 2004). The present study aimed to identify and characterize species within the SFG rickettsiae using sequence and phylogenetic analysis of ticks collected from humans, domestic and wild animals, and unfed (questing) ticks from the ground in distinct localities in Turkey.

Materials and methods

In 2011, ticks were collected from humans, domestic and wild animals, and from the ground as unfed (questing) ticks in 12 different provinces of Turkey: Ağrı, Ankara, Artvin, Bolu, Çankırı, Çorum, Erzurum, Giresun, Kırşehir, Kocaeli, Mardin, and Yozgat. Tick species, hosts, and tick localities are shown in Table 1. Host-seeking (questing) ticks were collected by making some vibrations on the ground. Ticks were identified according to the taxonomic keys of Apanaskevich (2003) and Estrada-Peña et al. (2004).

Each tick was first washed in 70% alcohol, then rinsed in sterile water, and dried on sterile filter paper. Ticks were individually homogenized using liquid nitrogen, and DNA was individually extracted by using the Qiagen DNeasy® blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Rickettsial DNA was detected by PCR using the primers *Rp* CS.409d and *Rp* CS.1258n, which amplify the citrate synthase gene (*gltA*) of *Rickettsia* spp. (Roux et al., 1997). Each tick positive for *gltA* was also tested for the *ompA* gene of *Rickettsia* spp. using the primers Rr. 190.70 and Rr. 190.701 (Fournier et al., 1998). DNase-RNase-free water was used as a negative control, and a positive control (DNA from *Ri. montanensis*) was included in all reactions. Successfully

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Table 1
Tested tick species collected from different localities in Turkey: their hosts, PCR positivity, and targeted gene.

Tick species (no. of tested specimens)	Provinces and numbers of the ticks collected	Host	No. of PCR-positive ticks
Hyalomma marginatum (31)	Ankara (15)	Human (6M,2F)	1M
		Cattle (4F, 1M)	0
		Sheep (1M)	0
		Questing tick (1M)	0
	Kırşehir (10)	Cattle (8M, 1F)	2M
		Questing tick (1F)	0
	Bolu (3)	Cattle (3M)	0
	Çorum (1)	Human (1M)	0
	Çankırı (1)	Human (1M)	0
	Erzurum (1)	Cattle (1F)	0
Hyalomma aegyptium (23)	Ankara (18)	Human (3M)	0
		Tortoise (5M, 5F)	1M, 1F
		Questing ticks (3F, 2M)[a]	1M, 1F
	Yozgat (5)	Tortoise (4M, 1F)	0
	Kırşehir (1)	Questing tick (1F)	0
Hyalomma excavatum (7)	Ankara (7)	Human (3M, 2F)	1M
		Cattle (2M)	0
Hyalomma scupense (2) (syn. *Hy. detritum*)	Ankara (2)	Cattle (2M)	0
Hyalomma spp. (22)	Ankara (22)	Human (20N)	3N
		Buzzard (2N)	0
Dermacentor marginatus (10)	Ankara (8)	Cattle (4M, 1F)	4M, 1F
		Human (3M)	3M
	Bolu (2)	Cattle (1M)	1M
		Questing tick (1M)	0
Haemaphysalis parva (11)	Ankara (11)	Human (8M, 3F)	2F, 2M
Rhipicephalus sanguineus (2)	Ankara (2)	Buzzard (1F, 1M)	0
Rhipicephalus turanicus (5)	Ankara (2)	Sheep (1F)	1F
		Cattle (1F)	0
	Kırşehir(3)	Cattle (2F, 1M)	0
Rhipicephalus bursa (1)	Ankara (1)	Cattle (1M)	0
Ixodes ricinus (4)	Kocaeli (1)	Human (1F)	0
	Giresun (1)	Human (1F)	0
	Artvin (1)	Human (1F)	0
	Yozgat (1)	Vole (1F)	0
Argas persicus (2)	Mardin (2)	Chicken (2F)	0
Argas spp. (5)	Mardin (5)	Chicken (5N)	0
Otobius megnini (1)	Ağrı (1)	Human (1F)	0
Total	126	63M (50%), 36F (28.5%), 27N (21.4%)	25 (19.8%)

F, female; M, male; N, nymph.
gltA, citrate synthase A gene; *ompA*, outer membrane protein A gene. Only ticks positive for the *gltA* gene were tested for the *ompA* gene.
[a] Four ticks collected from humans and one tick collected from buzzard were obtained as engorged nymph and were then allowed to molt to the adult stage.

amplified product was purified using the QIAquick® Extraction Kit (Qiagen GmbH). Purified DNA was sequenced using BigDye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Automated fluorescence sequencing was performed with an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems).

Nucleotide sequences were processed using nucleotide BLAST (National Center for Biotechnology Information, www.ncbi.nlmn.nih.gov/BLAST). Sequences were edited and aligned by using BioEdit software (Hall, 1999). Phylogenetic and molecular evolutionary analyses were performed by using MEGA version 4 (Tamura et al., 2007). The phylogenetic tree was produced by applying the Bootstrap Test of Phylogeny, Neighbor-Joining technique. The sequence data obtained in this study have been deposited in GenBank under the accession numbers JQ691710 to JQ691734.

Results

A total of 126 ticks representing 12 species, 7 genera, and 2 families were collected. Eight (6.3%) were argasid ticks, and 118 (93.7%) were ixodid ticks. Collected ticks belonged to the following species: *Hyalomma marginatum* ($n = 31$), *Hy. aegyptium* ($n = 23$), *Hy. excavatum* ($n = 7$), *Hy. scupense* (syn. *Hy. detritum*) ($n = 2$), *Hyalomma* spp. ($n = 22$), *Dermacentor marginatus* ($n = 10$), *Haemaphysalis parva* ($n = 11$), *Rhipicephalus sanguineus* ($n = 2$), *Rh. turanicus* ($n = 5$), *Rh. bursa* ($n = 1$), *Ixodes ricinus* ($n = 4$), *Argas persicus* ($n = 2$), *Argas* spp. ($n = 5$), and *Otobius megnini* ($n = 1$). Ticks were collected from humans ($n = 56$), from wild and domestic animals, including cattle (*Bos taurus*) ($n = 26$), sheep (*Ovis aries*) ($n = 2$), a vole (*Microtus* sp.) ($n = 1$), tortoises (*Testudo graeca*) ($n = 5$), buzzards (*Buteo rufinus*) ($n = 2$), chickens (*Gallus gallus domesticus*) ($n = 2$), and unfed ticks from the ground. The DNA of *Rickettsia* spp. was found in 25 ticks by using PCR and primers specific for the *gltA* gene. Twenty-five ticks positive for the *gltA* gene were tested for the *ompA* gene, and 4 ticks were found negative while 21 ticks were found positive. Twenty-one ticks yielded amplicons for both the *gltA* and the *ompA* gene; however, a PCR product of the *ompA* gene was not obtained in 4 *Ha. parva* species. Further information is summarized in Table 1. *OmpA* gene sequence analyses indicated that *Ri. aeschlimannii* was detected in 8 tick individuals (32%): 3 *Hy. marginatum* (collected from cattle and humans), 2 *Hy. aegyptium* (collected from humans as engorged nymphs; later the nymphs molted to the adult stage under the suitable conditions), 2 *Hyalomma* spp. (nymphs collected from humans), and 1 *Rh. turanicus* (from sheep). *Ri. africae* was found in 4 ticks (16%): 2 *Hy. aegyptium* (from tortoise), 1 *Hy. excavatum* (from human), and 1 *Hyalomma* sp. (nymph from human). Whilst 8 *D. marginatus* (32%) (from cattle and humans) were found infected with *Ri. slovaca*, only one *D. marginatus* (4%) (from cattle) was found infected with *R. raoultii*. *GltA* gene sequence analyses indicated that *Ri. hoogstraalii* occurred in 4 *Ha. parva* (16%) (from humans). None of the *Hy. scupense* (syn. *Hy. detritum*), *Rh. sanguineus*, *Rh. bursa*, *I. ricinus*, *Ar. persicus*, *Argas* spp., or *O. megnini* ticks