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Molecular identification of tick-borne bacteria in wild animals and their ticks in Central Anatolia, Turkey

Ömer Orkun*, Ayşe Çakmak

Department of Parasitology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

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ABSTRACT

Wild animals fulfill an important mission in the ecology of tick-borne diseases as both suitable hosts to tick vectors and reservoirs of the pathogens. However, current data regarding the role of wild animals in the ecology of tick-borne pathogens is insufficient and more investigations are required. In this study, we investigated tick-borne bacterial pathogens in wild boar, hare, and fox and their ticks in Turkey. A total of 102 tick pools comprised of 445 ticks and blood samples were analyzed for the presence of bacterial DNA by PCRs targeted rickettsial *gltA* and *ompA* genes, *5S-23S rDNA* gene for *Borrelia* spp., and *msp4* gene for *Anaplasma* spp. As a result of PCR and sequence analyses, three pathogenic spotted fever group (SFG) rickettsiae, two SFG rickettsiae with unknown pathogenicity and one pathogenic *Borrelia burgdorferi* sensu lato were detected in samples obtained from wild animals. *Rickettsia slovaca* was detected in ticks (13.7% of tick pools) collected from wild boars and blood of a wild boar. In addition, the presences of *R. hoogstraalii* (19.6% of tick pools), *R. aeschlimannii* (5.8% of tick pools), *R. sibirica* subsp. *mongolitimonae* (1.9% of tick pools) and *Candidatus* R. goldwasserii (0.9% of tick pools) were detected in ticks collected from wild animals. Furthermore, *B. burgdorferi* sensu stricto was detected in a tick pool collected from a wild boar. This is the first report on the presence of *Candidatus* R. goldwasserii in Turkey. Consequently, this study shows that pathogenic *Rickettsia* and *Borrelia* species are circulating in Turkish wildlife and these pathogens can pose a threat to human health. Also, it has been determined that the investigated wild animals play a role as maintenance host for vector ticks; therefore, these animals must also be considered in the ecology of the mentioned pathogens.

1. Introduction

Ticks that are ectoparasites of vertebrate can be found in all terrestrial regions of the world. They feed by sucking blood from the vertebrates and, in this way; transmit microorganisms that can cause diseases in humans and animals [1]. Ticks are recognized as the most important vectors of pathogens causing diseases in animal health and the second most important disease vectors in human health after mosquitoes [2,3]. In the last 30 years, many tick-borne disease outbreaks have occurred and new pathogens have been identified. Concurrently, new epidemiological and ecological data have been obtained regarding tick-borne diseases as a result of the development of molecular methods. However, further studies related to ticks and tick-borne pathogens are still needed [4,5].

Tick-borne bacterial pathogens cause significant diseases in humans and animals. The most common of these are the diseases caused by SFG rickettsiae, *Borrelia* spp., and *Anaplasma* spp. [2,6,7]. Ticks play an important role in the ecology of these diseases. Wild animals also play a key role in the ecology of tick-borne diseases and pose a threat to humans and domestic animals. Therefore, wildlife is an important factor in the control of these diseases [4,8]. In particular, wild mammals serve as suitable hosts in the life cycle of ticks in nature. In fact, certain life stages of some tick species are directly connected with wild animals. For example, immature *Hyalomma marginatum,* a two-host tick and the main vector of Crimean-Congo Hemorrhagic Fever virus [9] and *Rickettsia aeschlimannii* [10], feed on medium-sized mammals (e.g. hare, hedgehog, etc.) and ground-feeding birds (e.g. partridge, rook, etc.) [9]. In addition, wild boar is one of the prominent hosts of *H. marginatum* adults in wildlife [11]. Additionally, wild boars serve as suitable hosts for adult *Dermacentor marginatus*, a three-host tick [12] and primary vector of *Rickettsia slovaca* and *Rickettsia raoulti* [13]. This host mission of wild boars for *D. marginatus* in wildlife is most prominent during the late autumn and winter months when livestock are generally enclosed [11]. Furthermore, the adults of both tick species frequently attach to humans [14].

Wild animals play an important role in the life cycle of tick-borne

* Corresponding author.
E-mail address: omerorkun@yahoo.com.tr (Ö. Orkun).

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pathogens by serving as both suitable hosts and reservoirs [4]. However, this role is not completely understood in Turkey. Therefore, we aimed to investigate the presence of *Rickettsia* spp., *Borrelia* spp., and *Anaplasma* spp. in wild boars, hares, and foxes and their ticks. We also partially sought to determine the potential role of aforementioned wild animals in the ecology of tick-borne bacterial pathogens in Turkey.

2. Materials and methods

2.1. Sample collection, morphological identification of ticks and further biological processes

This study was conducted between 2013 and 2016 in Ankara province of Turkey. Study materials (blood samples and ticks) were collected from wild boars (*Sus scrofa*), hares (*Lepus europaeus*) and red foxes (*Vulpes vulpes*). Blood samples collected in EDTA- containing tube were taken directly from the heart shortly after (within few minutes) the animals were shot by hunters during the official hunting seasons. All animals were examined for the presence of ticks, which were subsequently collected. The samples (blood and ticks) were transferred under suitable conditions to the Protozoology and Entomology Laboratory of Ankara University for further processing. Blood samples were stored at −20 °C until DNA extraction. Live ticks were immediately processed upon arrival at the laboratory.

All collected ticks were identified morphologically using taxonomic keys [12,15,16]. Adult ticks and unfed larvae (that hatched in the laboratory from known female ticks) were identified at the species level, whereas nymphs were identified at the genus level to avoid misidentification due to lack of distinguishing features. Engorged nymphs (*Hyalomma* spp.) obtained from hares were incubated under suitable conditions (28 °C and 80–85% relative humidity) to allow them to develop into the adult stage (as unfed ticks). Subsequently, the unfed adult ticks were identified at the species level. In addition, a fully engorged female tick (*D. marginatus*) collected from a wild boar was maintained alive in an incubator under suitable conditions (22 °C and 80–85% relative humidity) until it oviposited and larvae hatched. Following morphological identification, all ticks were stored at −80 °C until DNA extraction.

Animal protocols were approved by the Animal Ethics Committee of Ankara University under register number 2013-17-127. Additionally, the required permission to conduct research on wild animals was obtained from the General Directorate of Nature Conservation and National Parks, Ministry of Forestry and Water Affairs with permission number 72784983-488.04-240315.

2.2. Molecular analyses (DNA extraction, PCR, sequencing, and phylogenetic analyses)

The obtained ticks were separated and pooled according to the degree of blood sucking, body size, and developmental stage. Each pool included the same species gathered from the same individual host. Ticks were pooled into groups consisting of 1–40 ticks. Each tick was first washed in 70% alcohol, and then rinsed in sterile distilled water before being dried on sterile filter paper to avoid environmental contamination. Ticks were homogenized by using a SpeedMill PLUS homogenizer (Analytikjena, Jena, Germany) according to the manufacturer's instructions. Genomic DNA was extracted from homogenized ticks using a BlackPREP tick DNA/RNA kit (Analytikjena) following the manufacturer's protocol. Extracted DNA was stored at −20 °C until PCR analysis.

Genomic DNA was extracted from anti-coagulated whole blood taken from wild animals using a DNeasy® blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA extracts were stored at −20 °C until PCR analysis.

Ticks and blood samples were screened for the presence of bacterial DNA (*Rickettsia* spp., *Borrelia* spp., and *Anaplasma* spp.). Rickettsial DNA was detected by conventional PCR with the primers *Rp* CS.409d and *Rp* CS. 1258n, which amplify a fragment of the citrate synthase gene (*gltA*) of *Rickettsia* spp. (common to the whole *Rickettsia* genus) [17]. Subsequently, *gltA* positive samples were also tested for the outer membrane protein A gene (*ompA*) of *Rickettsia* spp. using the primers Rr. 190.70 and Rr. 190.701, allowing the differentiation of closely-related strains [18]. For the detection of *B. burgdorferi* sensu lato species, a nested PCR, which amplifies a fragment of the *5 S–23 S rDNA* intergenic spacer (IGS), was performed using two sets of primers (RIS1-RIS2 and RIS3-RIS4) [19,20]. For the detection of *Anaplasma* spp., two conventional PCRs, which amplify fragments of the major surface protein 4 gene (*msp4*), were carried out using two sets of primers (*A. marginale/A. ovis*: MSP45-MSP43 primers and *A. phygocytophilum*: MAP4AP5-MSP4AP3 primers) [21]. Negative controls (Sterile DNase-RNase-free water) were used to check for sample contamination during handling and positive controls (DNA from *R. aeschlimannii*, *B. burgdorferi* sensu lato, *A. phygocytophilum,* and *A. ovis*) were included in all PCR runs. Pre-PCRs were also performed with positive controls at different dilutions (1-1/100) to avoid false negative results that may occur due to low copy numbers of bacterial genes.

The successfully amplified products were purified using a QIAquick® Gel Extraction Kit (Qiagen) following the manufacturer's protocol. Purified DNA was bi-directionally sequenced using a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) in accordance with the manufacturer's protocol. Automated fluorescence sequencing was performed with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Nucleotide sequences were compared with registered GenBank sequences using BLAST analysis (www.ncbi.nlmn.nih.gov/BLAST). The sequences were edited and aligned using BioEdit software [22]. jModeltest version 0.1.1 was used to determine the most appropriate model for our data [23]. Phylogenetic analyses were performed using MEGA version 7.0 [24]. The nucleotide sequences obtained in this study were deposited in GenBank under the accession numbers MF379268 to MF379312.

3. Results

A total of 30 wild boars, 30 hares, and four foxes were examined for tick infestations and almost all of them were subjected to blood collection. Blood samples were obtained from 26 wild boars. Tick samples alone were collected from four wild boars that we could not take blood samples. Of the 30 wild boars, 21 were determined to be infested by ticks. Both blood and tick samples were obtained from 17 wild boars. Blood samples were collected from all hares and of these, 18 were infested with ticks. Blood samples were collected from all foxes and tick infestation was detected in only one fox.

A total of 445 tick samples were collected from the targeted wild animals during the study; 214 ticks from 21 wild boars, 212 ticks from 18 hares, and 19 ticks from one fox. As a result of morphological identification, obtained ticks were determined to be *Haemaphysalis parva* (n = 162), *Hyalomma* spp. (nymph) (n = 120), *D. marginatus* (n = 62), *Rhipicephalus turanicus* (n = 30), *H. marginatum* (n = 27), and *H. excavatum* (n = 5). Ticks collected from wild boars were identified as *D. marginatus* (n = 62), *Ha. parva* (n = 57), *H. marginatum* (n = 27), *Rh. turanicus* (n = 24), and *H. excavatum* (n = 5). One of the collected *D. marginatus* was a fully engorged female and this tick was incubated under suitable conditions for egg production. After hatching, 40 unfed larvae were randomly selected and included in a pool. Ticks collected from hares were identified as *Hyalomma* spp. (nymph) (n = 120), *Ha. parva* (n = 86), and *Rh. turanicus* (n = 6). Of the obtained nymphs, 20 were engorged and they were incubated under suitable conditions to molt into the adult stage. All nymphs hatched successfully and became unfed-adults. After hatching, unfed adults were identified as *H. marginatum* (n = 19) and *H. aegyptium* (n = 1). All ticks collected from the fox were identified as *Ha. parva* adults (Table 1).

A total of 102 tick pools comprised of 445 ticks were created and

Table 1
Tick species collected from wild animals and PCR positivity.

Hosts	Tick species	No. of tested tick	No. of pools	PCR Positivity		
				No. of *Rickettsia*-positive pools (%)	No. of *Borrelia*-positive pools (%)	No. of *Anaplasma*-positive pools (%)
Wild boar	*D. marginatus*	101 (27 F, 34 M, 40 L[a])	21	14 (66.6%)	0	0
	Ha. parva	57 (6 F, 51 M)	9	9 (100%)	0	0
	H. marginatum	27 (10 F, 17 M)	10	3 (30%)	0	0
	Rh. turanicus	24 (10 F, 14 M)	5	1 (20%)	1 (20%)	0
	H. excavatum	5 (2 F, 3 M)	2	0	0	0
Hare	*Hyalomma* spp.	100 (N)	12	4 (33.3%)	0	0
	Ha. parva	86 (30 F, 56 M)	16	7 (43.7%)	0	0
	H. marginatum	19[b] (14 F, 5 M)	19	1 (5.2%)	0	0
	Rh. turanicus	6 (3 F, 3 M)	4	0	0	0
	H. aegyptium	1[b] (1 M)	1	0	0	0
Fox	*Ha. parva*	19 (9 F, 10 M)	3	3[c](100%)	0	0
Total		**445 (111 F, 194 M, 100 N, 40 L)**	**102**	**42 (41.1%)**	**1 (0.9%)**	**0**

F, female; M, male; N, nymph; L, larvae.
[a] The fully engorged female tick obtained from host was maintained alive in an incubator until egg production and larvae hatched, subsequently unfed larvae were subjected.
[b] The ticks were obtained as engorged nymphs from host and were then allowed them to molt into the adult stage (as unfed).
[c] One pool was founded to be infected as mixed with two Rickettsial DNA.

analyzed for the presence of bacterial DNA. By PCR analysis, we detected the presence of *Rickettsia* spp. in 42 tick pools (41.1%) and also *Borrelia* spp. in one tick pool (0.9%). In contrast, the presence of *Anaplasma* spp. was not detected in tick pools (Table 1). As a result of the blood sample PCR analyses, DNA of *Rickettsia* spp. was detected in one sample obtained from a wild boar. No DNA belonging to *Borrelia* spp. and *Anaplasma* spp. were detected in obtained blood samples. According to the sequence analyses; *R. slovaca* was detected in 13 *D. marginatus* and one *Rh. turanicus* pools obtained from 10 wild boars (Of these, each of the three wild boars had two positive tick pools, and one *D. marginatus* and *Rh. turanicus* positive pools were collected from same wild boar), and also in a blood sample obtained from a wild boar (tick samples collected from this wild boar were found negative for rickettsiae). BLAST analyses of the partial *ompA* gene indicated that the *R. slovaca* sequences (accession nos. MF379288-90, 92–98, MF379300, 03-05) obtained from ticks have 99.5–100% similarity with the sequences obtained from *D. marginatus* ticks in Italy (accession no. HM161787 and HM161776), *Melophagus ovinus* (the sheep ked) in China (accession no. KX506733) and the reference strain D-CWPP (accession no. CP003375), while the *R. slovaca* sequence (accession no. MF379311) obtained from blood of a wild boar is 100% similar to a sequence obtained from *D. marginatus* in Italy (accession no. HM161769) and the reference strain D-CWPP. It has also been detected that *R. slovaca* sequences obtained from ticks and blood of a wild boar are identical. *R. aeschlimannii* was detected in three *H. marginatum* pools obtained from three wild boar, 2 *Hyalomma* spp. (nymph) obtained from two hares, and one unfed *H. marginatum* (this tick were originally obtained as an engorged nymph from a hare). BLAST analyses of the partial *ompA* gene indicated that the obtained *R. aeschlimannii* sequences (accession nos. MF379291, 99, MF379302, 06-08) are 99.8–100% similar to the sequence obtained from a *H. impeltatum* in Egypt (accession no. HQ335157). *R. sibirica* subsp. *mongolitimonae* was detected in two *Hyalomma* spp. (nymph) pools obtained from two hares. BLAST analyses of the partial *ompA* gene indicated that the obtained *R. sibirica* subsp. *mongolitimonae* sequences (accession nos. MF379301, 09) are 100% identical to a sequence previously obtained from *H. marginatum* in Turkey (accession no. KY513920) (Table 2 and Fig. 1). *R. hoogstraalii* was detected in nine *Ha. parva* and one *D. marginatus* pool obtained from eight wild boars (Of these, three positive *Ha. parva* pools were collected from same wild boar), seven *Ha. parva* pools obtained from seven hares, and three *Ha. parva* pools obtained from one fox. BLAST analyses of the partial *gltA* gene indicated that the *R. hoogstraalii* sequences (accession nos. MF379268-74, 76–87) obtained from *Ha. parva* ticks have 99.1–100% similarity with the sequence obtained from a *Ha. parva* in Turkey (accession no. JQ691712), while the sequence (accession no. MF379275) obtained from a *D. marginatus* is 98.7% similar to the sequence of *Ha. sulcata* obtained in Croatia (accession no. DQ081187) (Table 2 and Fig. 2). *Candidatus* Rickettsia goldwasserii was detected in one *Ha. parva* pool obtained from a fox. BLAST analysis of the partial *ompA* gene indicated that the *Candidatus* Rickettsia goldwasserii sequence (accession no. MF379310) is 100% identical to a sequence obtained from a *Ha. adleri* tick collected from a golden jackal in Israel (accession no. HM136928) (Table 2 and Fig. 1). Of these, *R. hoogstrallii* and *Candidatus* R. goldwasserii were found as mixed in a *Ha. parva* pool. Additionally, the presence of *B. burgdorferi* sensu stricto was detected in a *Rh. turanicus* pool obtained from a wild boar. BLAST analysis of the partial *5 S–23 S rDNA* gene indicated that the obtained *B. burgdorferi* sensu stricto sequence (accession no. MF379312) is 99.5% similar to a sequence obtained from a *D. marginatus* tick in China (accession no. KP400557) (Table 2 and Fig. 3).

Detailed information about the ticks and nucleotide similarities of obtained bacterial sequences in this study are given in Tables 1 and 2, respectively. The phylogenetic trees were constructed using *gltA* and *ompA* genes of *Rickettsia* spp. and the *5 S–23 S rDNA* gene of *Borrelia* spp. Phylogenetic trees constructed using the nucleotide sequences recorded in GenBank and newly obtained sequences in this study are illustrated in Figs. 1–3.

4. Discussion

The roles of wild animals in the ecology of tick-borne pathogens are largely unknown or poorly understood. The most important reason for this scarcity is the difficulty in obtaining samples from wild animals. Nevertheless, wild animals have always been involved in the tick, host, and pathogen triangle. The most important roles of wild animals as suitable hosts to vector ticks and pathogens reservoirs [4,8]. Tick-borne bacterial microorganisms are important in both human and animal health. In particular, Lyme disease, SFG rickettsiae, and anaplasmosis are top of the list [2,6,7]. Although the presence of *Borrelia* spp., *Rickettsia* spp. and *Anaplasma* spp. in ticks, humans, and animals have already been reported in Turkey [20,25–28], little information exists regarding to the role of wild animals in the ecology of these bacteria. Data regarding wild animals and their ticks are required to determine the dynamics of these microorganisms in a region [8].

Table 2
Rickettsia spp. and *Borrelia* spp. detected in this study and their level of nucleotide similarity with other isolates.

Detected pathogens		Tick species/Blood samples (No. positive pools/ samples)	Sequenced gene	Nucleotide identity percentage	GenBank accession no.
***Rickettsia* spp.**	*R. hoogstraalii*	*Ha. parva* (19)	*gltA*	99.1-100[a]	MF379268-74 MF379276-87
		D. marginatus (1)	*gltA*	98.7[b]	MF379275
	R. slovaca	*D. marginatus* (13)	*ompA*	99.5-100[c,d,e,f]	MF379288-90 MF379292 MF379294-98 MF379300 MF379303-05
		Rh. turanicus (1)	*ompA*	100[c]	MF379293
		Blood (1)	*ompA*	100[e,g]	MF379311
	R. aeschlimannii	*H. marginatum* (4)	*ompA*	99.8-100[h]	MF379291 MF379299 MF379302 MF379308
		Hyalomma spp. (nymph) (2)	*ompA*	100[h]	MF379306-07
	R. sibirica subsp. *mongolitimonae*	*Hyalomma* spp. (nymph) (2)	*ompA*	99.8-100[i]	MF379301 MF379309
	Candidatus R. goldwasserii	*Ha. parva* (1)	*ompA*	100[j]	MF379310
***Borrelia* spp.**	*B. burgdorferi* sensu stricto	*Rh. turanicus* (1)	*5S-23S rDNA*	99.5[k]	MF379312

[a] *Rickettsia hoogstraalii* strain TR/Orkun-H.parva164/Ankara accession no. JQ691712.
[b] *Rickettsia* endosymbiont of *Haemaphysalis sulcata* accession no. DQ081187.
[c] *Rickettsia slovaca* isolate Kuqa01 accession no. KX506733.
[d] *Rickettsia slovaca* strain WB2/Dm Pavullo accession no. HM161787.
[e] *Rickettsia slovaca* strain D-CWPP accession no. CP003375.
[f] *Rickettsia slovaca* strain WB3/Dm Pavullo accession no. HM161776.
[g] *Rickettsia slovaca* strain WB7/Dm Pavullo accession no. HM161769.
[h] *Rickettsia aeschlimannii* strain EgyRickHimp-El-Arish-16 accession no. HQ335157.
[i] *Rickettsia sibirica* subsp. *mongolitimonae* isolate Hma-Adana accession no. KY513920.
[j] *Candidatus* Rickettsia goldwasserii isolate B5 accession no. HM136928.
[k] *Borrelia burgdorferi* isolate Alashankou-65 accession no. KP400557.

In this study, sequence analyses revealed to the presence of three pathogenic *Ricketsia* species (*R. slovaca*, *R. aeschlimanni,* and *R. sibirica* subsp. *mongolitimonae*) and two species with unknown pathogenicity (*R. hoogstraalii* and *Candidatus* R. goldwasserii) in the ticks and one pathogenic *Ricketsia* species (*R. slovaca*) in a blood sample of a wild boar. Of these, *R. slovaca*, the etiological agent of scalp eschar associated with neck lymphadenopathy after a tick bite (SENLAT) [29], was detected in 13 *D. marginatus* and one *Rh. turanicus* pools obtained from wild boars and also in the blood of a wild boar. Our results show that wild boars act mostly an important role by serving as a suitable host for the main vector ticks (*D. marginatus*) in the ecology of *R. slovaca*, although the bacteremia has been detected in only one blood sample of a wild boar. In this study, the presence of *R. slovaca* was detected in 66.6% of all *D. marginatus* pools. This indicates that high infection rates exist in *D. marginatus* feeding on wild boars and this bacterium can pose a threat to the local human population. Similarly, recent studies conducted in the same area reported the presence *R. slovaca* DNA in 64–80% of all *D. marginatus* ticks collected from humans and cattle [26,27]. When the results are compared, *R. slovaca* is seen to circulate at high density in *D. marginatus* in the region. It is possible that wild boars are responsible for this situation because the wild boar population has increased dramatically in recent years due to an abundance of suitable habitats in Turkey [30]. Globally, *R. slovaca* has been reported in *D. marginatus* collected from wild boars in France [31], Spain [32,33], and Italy [34]. In addition, the presence of antibodies against *R. slovaca* has been reported in sera of wild boars in Northeastern Spain [33]. Although the detection of *R. slovaca* in the blood of a wild boar in our study confirms the role of these animals in the life cycle of *R. slovaca*, we could not detect a direct correlation between wild boars and the positive ticks. In other words, all *R. slovaca*-positive ticks were collected from wild boars whose blood samples were negative for this bacterium; on the other hand, ticks collected from a wild boar whose blood sample was *R. slovaca*-positive. Therefore, additional studies are required to investigate the relationship between *R. slovaca* and wild boar. Interestingly, *R slovaca* infection in humans has not yet been reported in Turkey. However, our results indicate that *R. slovaca* infection should be considered in humans with SENLAT syndrome and infested with *D. marginatus* ticks in Turkey.

The presence of *R. aeschlimannii* was detected in three *H. marginatum* pools collected from wild boars, two *Hyalomma* spp. (nymph) pools obtained from hares and one unfed *H. marginatum* (originally obtained from a hare as an engorged nymph). *R. aeschlimannii*, which causes febrile illness in humans, is mainly transmitted by *Hyalomma* ticks (especially *H. marginatum*) [10,13] The presence of this bacterium has previously been reported in ticks in Turkey [26,27,35–37]. Consistent with our study, it has been shown that *H. marginatum* plays a primary role in the transmission of *R. aeschlimannii* [10,13]. Furthermore, our study indicates that wild boars and hares function in the life cycle of this bacterium by serving as suitable hosts to adult and immature *H. marginatum*, respectively. Additionally, the presence of *R. aeschlimannii* in unfed *H. marginatum* indicates that transstadial transmission occurs in this tick species. All of the data indicate that *R. aeschlimannii* is circulating in *H. marginatum* in Turkish wildlife. Although *R. aeschlimannii* infection has not been reported in humans, this disease should be taken into consideration in patients with *H. marginatum* tick bites in Turkey.

R. sibirica subsp. *mongolitimonae*, another pathogenic rickettsiae, was detected in two *Hyalomma* spp. (nymph) pools collected from hares. *R. sibirica* subsp. *mongolitimonae*, the etiological agent of lymphangitis-associated rickettsiosis (LAR), is mainly transmitted by ticks belonging to the genus of *Hyalomma* and *Rhipicephalus* [13]. In Turkey, this bacterium has been reported in *H. marginatum* collected from a patient with rickettsial infection [38] and *H. marginatum* ticks obtained from humans [36]. Here, the presence of this bacterium was detected in *Hyalomma* nymphs. Although the nymphs were identified at the genus level, 19 of the 20 nymphs collected from hares were identified as *H.*

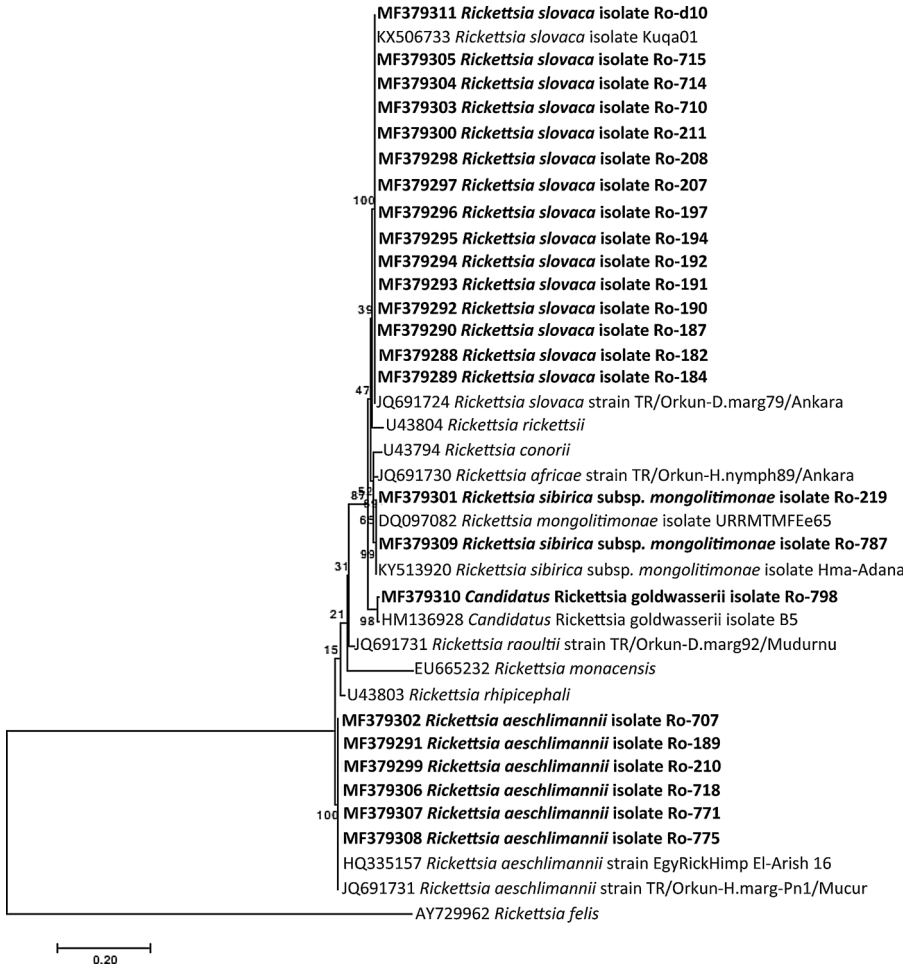

Fig. 1. Phylogenetic tree based on aligned sequences of the rickettsial *ompA* gene and constructed by using Maximum Likelihood method calculated under the GTR + G substitution model. The rickettsial sequences obtained in this study are shown in bold. GenBank accession numbers of sequences and names of lineages are given before species names.

marginatum after hatching. This result shows that the positive nymphs are most likely *H. marginatum*. The current study indicates that *R. sibirica* subsp. *mongolitimonae* is circulating in Turkey and should be taken into consideration in patients infested with *Hyalomma* nymphs. Additionally, this study shows that hares can play an important role in the life cycle of this bacterium by serving as suitable hosts for immature *Hyalomma* ticks.

The presence of *R. hoogstraalii*, which lacks precise data regarding pathogenesis, was detected in nine *Ha. parva* and one *D. marginatus* pools collected from wild boars, seven *Ha. parva* pools collected from hares and three *Ha. parva* pools collected from a fox. The pathogenicity of *R. hoogstraalii* is not yet known [13]. In Turkey, *R. hoogstraalii* has been reported in *Ha. parva* ticks [26,27,37]. In this study, the presence of this bacterium was detected in *Ha. parva* ticks collected from all inspected wild animal species. This study shows that *R. hoogstraalii* exists in this area and circulates between *Ha. parva* ticks. Additionally, detailed studies are needed to determine the pathogenicity of this SFG rickettsia.

Candidatus R. goldwasserii, uncultured SFG rickettsiae, was detected in a *Ha. parva* pool collected from a fox. This bacterium was detected in combination with *R. hoogstraalii* in the same pool. *Candidatus* R. goldwasserii was first detected in *Haemaphysalis* ticks (*Ha. parva* and *Ha. adleri*) collected from golden jackals in Israel [39]. Subsequently, the presence of this bacterium was reported in *Ha. adleri, Ha. parva, Rh. turanicus* and *Rh. sanguineus* ticks collected from domestic animals in Palestine [40]. *Candidatus* R. goldwasserii has not been associated with any disease to date. In this study, this bacterium was detected in a *Ha. parva* tick and to the best of our knowledge, this is the first detection of *Candidatus* R. goldwasserii in ticks in Turkey and only the third such report in the world. Our study, consistent with the others [39,40], indicates that wild carnivores could be a suitable reservoir for this bacterium, but this hypothesis needs to be confirmed by future field or experimental studies.

In this study, while the prevalence of *Rickettsia* spp. in tick pools was 41.1%, only one animal blood sample was founded as positive for rickettsiae. These situation shows that the investigated wild animals in the study region act mostly maintenance host role for the *Rickettsia*-infected ticks rather than reservoir host role. At this stage, the additional field and experimental studies are required to determine the reservoir role of wild animals in the ecology of tick-borne rickettsial diseases.

Borrelia burgdorferi sensu stricto was detected in a *Rh. turanicus* pool collected from a wild boar. *B. burgdorferi* sensu stricto, the etiological agent of Lyme disease in humans, is transmitted mainly by *Ixodes* spp. (e.g. *I. ricinus*) [7]. Interestingly, this bacterium was detected in an unusual tick species (*Rh. turanicus*). However, the blood sample from the positive tick-infested wild boar was negative for *Borrelia* by PCR. This indicates that the tick could have acquired the pathogen during a previous life stage. Of course, this data cannot provide exact details about the vector competence of this tick based on the current data. However, *B. burgdorferi* sensu stricto has been reported in *H. marginatum, H. excavatum, Hyalomma* spp. (nymph) and *Ha. parva* ticks collected from humans in a previous study conducted in the same region of Turkey [27]. Together, these data imply that this bacterium can use

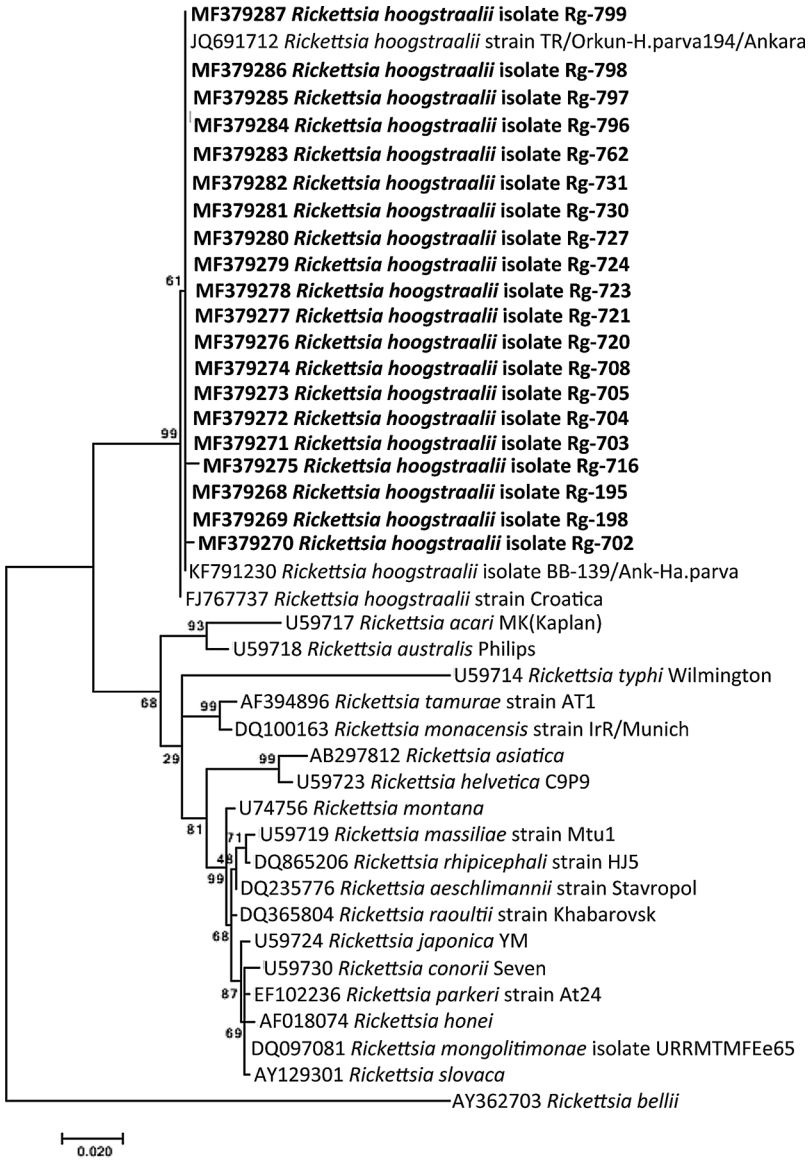

Fig. 2. Phylogenetic tree based on aligned sequences of the rickettsial *gltA* gene and constructed by using Maximum Likelihood method calculated under the GTR + I + G substitution model. The rickettsial sequences obtained in this study are shown in bold. GenBank accession numbers of sequences and names of lineages are given before species names.

different vectors in this area. However, detailed experimental and field studies are required at this stage. Additionally, the results of this study have confirmed that *B. burgdorferi* sensu stricto is circulating in this area and can pose a risk to the local human health.

In contrast to *Rickettsia* and *Borrelia* spp., *Anaplasma* spp. could not be detected in any samples in this study. *Anaplasma* spp. containing pathogens of domestic animals and humans are of medical and veterinary importance [6]. This study shows that mentioned wild animals play little or no role in the life cycle of *Anaplasma* species in the study area. Similarly, a recent study conducted in the same region showed that *Anaplasma* spp. was not detected in blood and tick samples of Anatolian wild sheep [41]. However, further investigation is necessary to determine the role of wild animals in the transmission of *Anaplasma*.

5. Conclusions

This study demonstrates the potential role of the investigated wild animals in the partial ecology of some tick-borne bacteria in Turkey. Human pathogenic SFG rickettsiae were detected in this study. Of these, *R. slovaca* was detected both in ticks and blood from a wild boar, therefore revealing the partial ecology of this pathogen in wildlife. In addition, the presence of two other pathogenic SFG rickettsiae (*R. aeschlimannii* and *R. sibirica* subsp. *mongolitimonae*) is shown in ticks obtained from wild animals. This study reveals that wild animals can play an important role in the ecology of these pathogens especially by serving as suitable hosts to vector ticks. Moreover, the presence of *R. hoogstraalii* and *Candidatus* R. goldwasserii, which are of unknown pathogenicity, is shown. This is the first report on the presence of *Candidatus* R. goldwasserii in Turkey. Additionally, the presence of *B. burgdorferi* sensu stricto, which is a pathogenic species, is shown in an unusual tick species. Consequently, our results reveal that the pathogenic tick-borne bacteria are circulating in Turkish wildlife and this situation can pose a threat to human health. Also, this study has determined that the investigated wild animals play a role as maintenance host for vector ticks; therefore, these animals must also be considered in the ecology of the mentioned pathogens.

Conflict of interests

The authors declare that they have no conflict of interest.

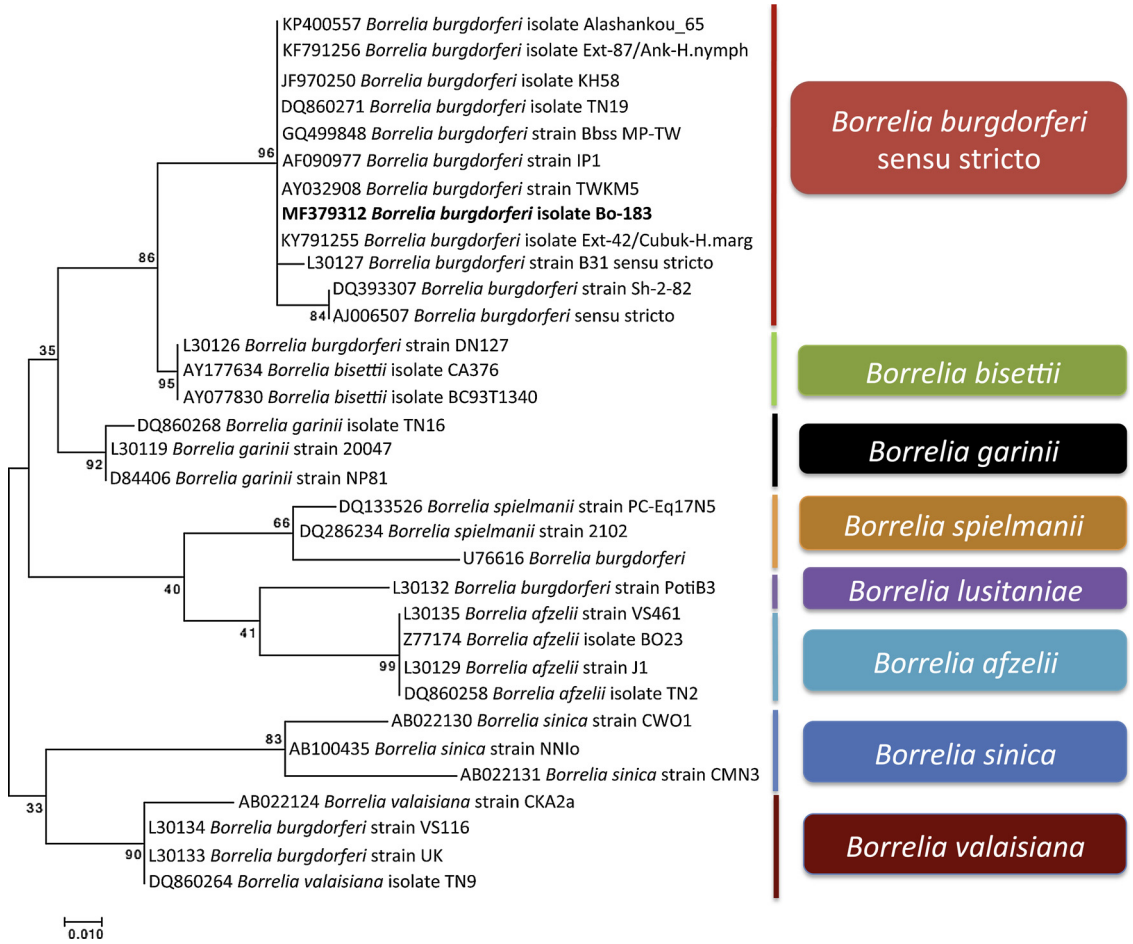

Fig. 3. Phylogenetic tree based on aligned sequences of *5 S–23 S rDNA* intergenic spaces region of *Borrelia burgdorferi* sensu lato and constructed by using Maximum Likelihood method calculated under the GTR + I+G substitution model. The *Borrelia* sequence obtained in this study is shown in bold. GenBank accession numbers of sequences and names of lineages are given before species names.

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