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Araştırma / Original Paper

Presence of the Zoonotic *Borrelia burgdorferi* sl. and *Rickettsia* spp. in the Ticks from Wild Tortoises and Hedgehogs

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ÖZET

Doğal yaşamdaki kaplumbağa ve kirpilerin kenelerinde zoonotik *Borrelia burgdorferi* sl. ve *Rickettsia* spp. varlığı

Amaç: Keneler tarafından bulaştırılan Riketsioz ve Borelyoz önemli zoonotik hastalıklardır. Çalışmada bu hastalıkların etkenleri Trakya yöresinde doğal alanlarda saptanan kaplumbağa ve kirpilerden toplanan kenelerde araştırılmıştır.
Yöntem: Toplam 438 kene larva, nimf ve erişkini nested-PCR yöntemi ile etkenler yönünden taranmıştır.
Bulgular: Oluşturulan 28 örnek grubundan 8 tanesi sadece *Rickettsia* spp., 2 tanesi de *Rickettsia* spp. ve *Borrelia burgdorferi* sl. ile enfekte bulunmuştur. Kirpilerden toplanan kenelerde sadece *Rickettsia* spp. pozitifliği saptanmıştır.
Sonuç: Çalışmanın sonuçları, doğal yaşamdaki kaplumbağa ve kirpilerin zoonotic hastalıklar açısından dikkate alınması ve izlenmesi gereğini vurgulamaktadır.
Anahtar sözcükler: *Borrelia burgdorferi* sl., *Rickettsia* spp., kaplumbağa, kirpi, Türkiye, Zoonoz

ABSTRACT

Presence of the zoonotic borrelia Burgdorferi sl. and *Rickettsia* spp. in the ticks from wild tortoises and hedgehogs

Objective: Rickettsiosis and borreliosis are two important tick-borne diseases. The presence of causative agents of these diseases was investigated in ticks collected from wild tortoises and hedgehogs in Thrace region of Turkey.
Method: A total of 438 ticks of all life stages were screened for *Borrelia burgdorferi* s.l. and *Rickettsia* spp., by employing nested PCR.
Results: Of 28 pools of tortoise ticks, eigth pools gave positive results for only *Rickettsia* spp., while two pools for both *Rickettsia* spp. and *B. burgodferi* s.l. No pathogen was detected in four pools of hedgehog ticks, but one pool including I. ricinus nymphs was positive for *Rickettsia* spp.
Conclusion: The study revealed once again that tortoises and hedgehogs should be taken into account in terms of zoonotic diseases.
Key words: *Borrelia burgdorferi* sl., *Rickettsia* spp., tortoise, hedgehog, Turkey, Zoonosis

INTRODUCTION

One of the most important parasites of domesticated and wild animals, ticks show considerably variation in host preference based on species and life stages. Ticks which could not find a chance of contact with an appropriate host tend to feed on a host of different species, and this gives rise to transmission of disease agents, and accordingly spread of zoonotic diseases such as rickettsiosis and Lyme borreliosis (1).

Rickettsiosis, known as one of the oldest arthropod-borne diseases, is an infection which is seen throughout the world and can cause deaths. The causative agent is obligate intracellular bacteria of the genus *Rickettsia* included in the bacterial family Rickettsiaceae. The genus consists of some 25 pathogenic species and numerous strains which have not been characterized yet (2). Species which cause rickettsiosis are grouped as typhus group, spotted fever group, transitional group which have characteristics of both typhus and spotted fever groups, ancestral group (non virulent) and scrub typhus group (3). Their transmissions are provided by several species of blood-sucking arthropods. However, 17 species within the spotted fever group are transmitted by ticks only. Ticks have also

been reported to harbour a vast number of symbiotic *Rickettsia* species pathogenity of which is not known in any detail. The most significant vector of rickettsiosis are ticks and at the same time they are reservoir for that disease. Studies indicate evidence of transstadial, cofeeding, venereal and transovarial transmission of the disease agents in ticks (2,4).

Borreliosis is caused by spirochete bacteria included in the genus *Borrelia*, family Spirochaetaceae. The disease is mainly considered as Lyme borreliosis and relapsing fever based on clinical symptoms in humans. Lyme borreliosis is the most common arthropod-borne disease seen in temperate zone of the northern hemisphere, and annually around 65.000 people suffer from this disease in Europe. *Borrelia burgdorferi* sensu lato (s.l.) complex which causes Lyme borreliosis is reported to comprise at least 18 genospecies. Pathogenities of these genospecies, the resulting clinical picture, and species of vertebrate host and vector ticks are different. However, vector ticks for transmission of Lyme borreliosis are clasified in the genus *Ixodes*. The causative agent is transmitted transtadially in the tick, while cofeeding (5,6) and incidental transovarial transmission (7) are reported to be possible.

The present study investigated the presence of pathogenic and zoonotic *Rickettsia* spp. and *Borrelia burgdorferi* s.l. in ticks collected from wild tortoises and hedgehogs in Thrace region of Turkey.

MATERIAL AND METHODS

Study area and material: This study was conducted on ticks collected from five hedgehogs (unidentified species) and 26 tortoises *(Testudo graeca)* in rural areas of Kirklareli, Tekirdag, Edirne and Istanbul in the years of 2009 and 2010. Removed from the animals with a pair of forceps, and cryopreserved, ticks were brought to the laboratory and

Table 1: Pools of the collected ticks and results of PCR screening

Location	Ticks					Hosts		PCR Results	
	Species	Male	Female	Nymph	Larva	Type	Number	Rickettsia spp.	Borrelia burgdorferi s.l.
Kirklareli	H. aegyptium	-	-	9	-	Hedgehog	1	-	-
Kirklareli	Hyalomma spp.	-	-	-	2	Hedgehog	1	-	-
Kirklareli	Haemaphysalis spp.	-	-	-	1	Hedgehog	1	-	-
Istanbul	H. aegyptium	-	-	2	-	Hedgehog	1	-	-
Istanbul	I. ricinus	-	-	1	-	Hedgehog	1	+	-
Istanbul	H. aegyptium	-	-	5	-	Tortoise	4	+	+
		7	-	-	-			+	-
		-	-	35	-			-	-
Istanbul	H. aegyptium	4	-	-	-	Tortoise	1	+	-
Istanbul	H. aegyptium	-	-	2	-	Tortoise	1	-	-
Kirklareli	H. aegyptium	8	10	-	-	Tortoise	1	-	-
Kirklareli	H. aegyptium	10	1	6	-	Tortoise	1	-	-
Kirklareli	H. aegyptium	-	-	2	18	Tortoise	1	-	-
Kirklareli	H. aegyptium	-	-	-	100	Tortoise	1	-	-
Kirklareli	H. aegyptium	-	2	-	-	Tortoise	1	+	-
Kirklareli	H. aegyptium	-	-	14	-	Tortoise	1	+	+
Kirklareli	H. aegyptium	2	-	-	-	Tortoise	1	-	-
Kirklareli	H. aegyptium	-	-	1	-	Tortoise	1	-	-
Kirklareli	H. aegyptium	8	-	-	-	Tortoise	1	+	-
Edirne	H. aegyptium	2	-	-	-	Tortoise	1	+	-
Edirne	H. aegyptium	1	-	-	-	Tortoise	1	-	-
Edirne	H. aegyptium	-	-	11	-	Tortoise	1	+	-
Edirne	H. aegyptium	-	-	1	-	Tortoise	1	-	-
Edirne	H. aegyptium	-	-	-	5	Tortoise	1	-	-
Edirne	H. aegyptium	-	-	31	-	Tortoise	1	-	-
Edirne	H. aegyptium	-	-	-	2	Tortoise	1	-	-
Tekirdag	H. aegyptium	13	5	-	-	Tortoise	1	-	-
Tekirdag	H. aegyptium	-	-	4	-	Tortoise	1	+	-
Tekirdag	H. aegyptium	-	-	-	2	Tortoise	1	-	-
		-	-	30	-		1	-	-
		-	-	-	35			+	-
Tekirdag	H. aegyptium	-	-	20	-	Tortoise		-	-
		-	-	26	-			-	-
Total		55	18	200	165		31	11	2

identified under a stereomicroscope (8,9).

Pooling of ticks: The collected ticks were grouped according to their hosts, species and life stages, and then were pooled for DNA extraction. As an exception, ticks from only one tortoise in Tekirdağ, which carried abundant nymphs and larvae, were pooled into four groups: three groups of nymphs including 20, 26 and 30 each, and a group of 35 larvae. Ticks from four tortoises in a location (Kucuk Sinekli Goleti/Silivri) in Istanbul were pooled into two groups of nymphs (5 and 35 nymphs in each group) and a group of seven adult ticks (Table 1) as well.

DNA extraction and nested-PCR: Ticks were decontaminated in 70% ethanol and sterile double distilled water, air-dried for a maximum of 10 minutes and then homogenized in liquid nitrogen. DNA was extracted with QIAamp DNA Mini Kit (10), and the obtained samples were stored at -80°C until use.

Semi nested-PCR using specific primers which were designed for pathogenic species of the relevant genera found in Europe was employed to screen samples for *Borrelia burgdorferi* s.l. and *Rickettsia* spp. OspA gene of *Borrelia burgdorferi* s.l. (Primers; OSPAFw1: ttgggaataggtctaatattagc, BorR: actaatgttttvccatcttc, OSPAFw2: atttcctggaagcttaatgc, ValR: atgyaagcaaaatgttagc, PCR conditions; first step: OSPAFw1-BorR, second step: OSPAFw2-BorR, 94°C/2 min (94°C/1 min, 50°C/1 min, 72°C/2 min) 45 cycles, 72°C/10 min) and citrate synthase (gltA) gene of *Rickettsia* spp. (Primers; RickF1: gggttttggtcatcgtgtat, RickR1: cccgaataaaaatcaacatt, RickR2: tctctcaataaaatattcatctttaag, PCR conditions; first step: Rick F1-RickR1, second step: Rick F1-RickR2, 95°C/2 min (95°C/30 s, 50°C/1 min, 72°C/1 min) 44 cycles, 72°C/5 min) were targeted.

RESULTS

A total of 423 ticks were obtained from 26 tortoises. All of the adults, nymphs and larvae (55 males, 18 females, 188 nymphs and 162 larvae) were identified as *Hyalomma aegyptium*. Fifteen ticks collected from five hedgehogs were as follows: six nymphs of *H. aegyptium*, two larvae of *Hyalomma* spp., one larva of *Haemaphysalis* spp. and six nymphs of *Ixodes ricinus*. Larvae could be identified to the genus level.

Of 28 pools of tortoise ticks, eight pools gave positive results for only *Rickettsia* spp., while two pools for both *Rickettsia* spp. and *B. burgodferi* s.l. No pathogen was detected in four pools of hedgehog ticks, but one pool including *I. ricinus* nymphs was positive for *Rickettsia* spp. (Table 1).

DISCUSSION

Ticks were reported to be primary reservoir for *Rickettsia*l species of the spotted fever group, and tick species such as *Rhipicephalus sanguineus, Dermacentor marginatus, D. reticulatus, Hyalomma asiaticum, H. marginatum, I. ricinus* are known to play a role in transmission. Factors such as survival of the parasite during the whole lifespan of the tick and its transovarial transmission make ticks a perfect reservoir (4,11). However, many domesticated and wild vertebrates can be infected, thus contributing to persistence of the relevant disease (12).

Concerning spotted fever group rickettsiosis in Turkey, studies including serologic screening indicated 11,7% and 13,7% seropositivity in populations at risk in Black Sea (13) and Mediterranean (14) regions, respectively. Serologically confirmed cases of Mediterranean spotted fever in Thrace region of Turkey, an endemic area for tick-borne diseases, revealed the presence of *Rickettsia conorii* subsp. conorii (15). Available studies are case reports and detailed epidemiologic studies on rickettsiosis are still lacking. Furthermore, no study is available in terms of animals which may play role in natural cycle of this disease.

In tick screening studies in our country, from 40,62 to 97,22 % of the tortoises were found to be infested with *H. aegyptium* (16-18), and a study covering Balkan countries revealed that *Hyalomma* spp., *R. sanguineus* and *Haemaphysalis inermis* also can infest tortoises (19). It is known that all life stages of *H. aegyptium*, primary tick of tortoises, can feed on tortoises, especially preferring adult ones (20), and attach to and feed on humans (21,22). In addition, this tick species can transmit some zoonotic agents such as *Rickettsia aeschlimannii* (23), Q fever agent, *Coxiella burnetii* (24), thus exposing a risk for spread of tick-borne diseases in other countries through tortoise trade (25). Present study indicated the existence of zoonotic *Rickettsia* spp. in all life stages of *H. aegyptium*. Moreover, our finding that the agent exists in 36 larvae, but does not in 76 nymphs collected from the same tortoise, confirms the possibility of transovarial transmission, as stated by Socolovschi et al (4).

In the present study, positivity for *Rickettsia* spp. was found in a *I. ricinus* nymph collected from a hedgehog. Several studies reported that hedgehogs serve as a host of many tick species, mainly *Ixodes hexagonus* and *I. ricinus* (26), carry zoonotic agents such as tick borne encephalitis virus (27), *Anaplasma phagocytophilum* (28), *B. burgdorferi* s.l. group (*B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. spielmanii*), and are reservoir for Lyme borreliosis (29). *B. burgdorferi* s.l. was not found in hedgehog ticks in our study, while two of 28 tortoise tick pools gave positivity. It is, however, clear that the number of hedgehogs investigated is quite limited to evaluate status of the disease in this species.

Agents of Lyme borreliosis are transmitted through ticks in the genus *Ixodes*, which can also serve as a reservoir for that disease. Nevertheless, some vertebrates which can be reservoir are important in the natural dynamics of the disease, such that several mouse species, rats, other small rodents, squirrels, hedgehogs, rabbits, red foxes, birds and some other animals serve as reservoir to varying degrees based on the species of the agent (5). Further on, some lizard species were recorded as reservoir for *Borrelia lusitaniae* (6).

In Turkey, Lyme borreliosis was identified in a patient in 1990 for the first time. Up to now, over 20 clinical case reports are available, but overlooked or unreported cases are likely to exist. Serologic screening revealed seropositivity varying between 7.8-44% in risky areas, and the risk was reported to be higher in Marmara and Black Sea regions where a dense population of *I. ricinus* ticks is present (30,31). Of *B. burgdorferi* s.l. group, *B. burgdorferi* sensu stricto, *B. garinii* (Eurasian type), *B. afzelii, B lusitaniae, B. valaisiana* (32) and *B. spielmani* (33) were detected in screening studies including *I. ricinus*. Seropositivity for *B. afzelii* was also found in wild Apodemus sylvaticus (34).

Some studies in Turkey revealed that certain numbers of tick species can attach to humans, especially *Ixodes ricinus*, which can infest varied animals including hedgehogs, and *Hyalomma aegyptium* known as tortoise tick (21,22). As a result of this study, tortoises and hedgehogs should be taken into account in terms of the transmission and natural niches of the zoonotic diseases.

REFERENCES

1. Jongejan F, Uilenberg G. The global importance of ticks. Parasitol. 2004;129:3-14.
2. Fournier PE, Raoult D. Current knowledge on phylogeny and taxonomy of Rickettsia spp. Ann NY Acad Sci. 2009;1166:1-11.
3. Gillespie JJ, Williams K, Shukla M, Snyder EE, Nordberg EK, Ceraul SM et al. Rickettsia phylogenomics: unwinding the intricacies of obligate intracellular life. PLoS One. 2008; 3(4): 2018.
4. Socolovschi C, Mediannikov O, Raoult D, Parola P. The relationship between spotted fever group Rickettsiae and Ixodid ticks. Vet Res. 2009; 40:34-50.
5. Piesman J, Gern L. Lyme borreliosis in Europe and North America. Parasitol. 2004; 129:191-220.
6. Rizzoli A, Hauffe HC, Carpi G, Vourc'h GI, Neteler M, Rosà R. Lyme borreliosis in Europe. Euro Surveill. 2011; 16(27):1-8.
7. Nefedova VV, Korenberg EI, Gorelova NB, Kovalevskii YV. Studies on the transovarial transmission of Borrelia burgdorferi sensu lato in the taiga tick Ixodes persulcatus. Folia Parasitol (Praha). 2004; 51(1):67-71.
8. Apanaskevich DA. To diagnostics of Hyalomma aegyptium (Acari: Ixodidae). Parazitologiya. 2003; 37:47-59.
9. Walker AR, Bouattour A, Camicas JL, Estrada-Peña A, Horak IG, Latif AA et al. Ticks of domestic animals in Africa. A guide to identification of species. Bioscience Reports, Scotland, UK. 2003
10. Sparagano O, Allsopp MTEP, Mank RA, Rijpkema SGT, Figuerova JV, Jongejan F. Molecular detection of pathogen DNA in ticks (Acari:Ixodidae). Exp Appl Acarol. 1999; 23:929-960.
11. Blanco JR, Oteo JA. Rickettsiosis in Europe. Ann NY Acad Sci. 2006; 1078:26-33.
12. Paddock CD. The science and fiction of emerging rickettsioses. Ann NY Acad Sci. 2009; 1166:133-143.
13. Tekin A, Gözalan A, Çöplü N, Yılmaz G, Köksal İ, Esen B, Ertek M. The seropositivity and risk factors for rickettsial infections in selected areas from Black Sea Region of Turkey. Dicle Med J. 2010 37(3):204-210.
14. Vural T, Ergin Ç, Kurşun AE. Antalya yöresinde Rickettsia conorii antikorlarının araştırılması. Mikrobiyol Bült. 1995; 29:370–374.
15. Kuloglu F, Rolain JM, Aydoslu B, Akata F, Tugrul M, Raoult D. Prospective evaluation of rickettsioses in the Trakya (European) region of Turkey and atypic presentations of Rickettsia conorii. Ann N Y Acad Sci. 2006; 1078:173-175.
16. Aydın L. Güney Marmara Bölgesinde görülen kene türleri ve yayılışları. T Parazitol Derg. 2000; 24(1):194-200.
17. Aydın L, Yıldırımhan HS, Uğurtaş İH. Marmara Bölgesi'ndeki bazı kertenkele ve kaplumbağa türlerinde kenelerin (Ixodidae) yaygınlığı. T Parazitol Derg. 2002; 26:84-86.

18. Aysul N, Kar S, Yılmazer N, Alp HG, Gargılı A. Trakya Yöresi'ndeki kaplumbağalarda (Testudo graeca) Hyalomma aegyptium (Lineaus, 1758)'un yaygınlığı. Pendik Vet Mikrobiol Derg. 2010; 37(1):53-56.

19. Siroky P, Petrzekova KJ, Kamler M, Mihalca AD, Modry D. Hyalomma aegyptium as dominant tick in tortoises of the genus Testudo in Balkan countries, with notes on its host preference. Exp Appl Acarol. 2006; 40:279-290.

20. Apanaskevich DA. Host–parasite relationships of the genus Hyalomma Koch (Acari, Ixodidae) and their connection with microevolutionary processes (in Russian). Parazitologiya. 2004; 38:515-523.

21. Gargılı A, Kar S, Yılmazer N, Cerit Ç, Sönmez G, Şahin F, Alp H, Vatansever Z. Evaluation of ticks biting humans in Thrace Province, Turkey. Kafkas Univ Vet Fak Derg. 2010; 16(Suppl-A):141-146.

22. Gargılı A, Kar S, Yılmazer N, Ergönül Ö, Vatansever Z. Different abundances of human-biting ticks in two neighboring provinces in Turkey. Kafkas Univ Vet Fak Derg. 2011; 17(Suppl A):93-97.

23. Bitam I, Kernif T, Harrat Z, Parola P, Raoult D. First detection of Rickettsia aeschlimannii in Hyalomma aegyptium from Algeria. Clin Microbiol Infect. 2009; 15(2):253-254.

24. Široký P, Kubelová M, Modrý D, Erhart J, Literák I, Špitalská E, Kocianová E. Tortoise tick Hyalomma aegyptium as long term carrier of Q fever agent Coxiella burnetii-evidence from experimental infection. Parasitol Res. 2010; 107:1515-1520.

25. Brianti E, Dantas-Torres F, Giannetto S, Risitano A, Brucato G, Gaglio G, Otranto D. Risk for the introduction of exotic ticks and pathogens into Italy through the illegal importation of tortoises, Testudo graeca. Med Vet Entomol. 2010; 24:336-339.

26. Gern L, Rouvinez E, Toutoungi LN, Godfroid E. Transmission cycles of Borrelia burgdorferi sensu lato involving Ixodes ricinus and/or I. hexagonus ticks and the European hedgehog, Erinaceus europaeus, in suburban and urban areas in Switzerland. Folia Parasitol (Praha). 1997; 44:309-314.

27. Labuda M, Randolph SE. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. Zentralbl Bakteriol. 1999; 289:513-524.

28. Skuballa J, Petney T, Pfaeffle M, Taraschewski H. Molecular detection of Anaplasma phagocytophilum in the European hedgehog (Erinaceus europaeus) and its ticks. Vector Borne Zoonot Dis. 2010; 10(10):1055-1057.

29. Skuballa J, Oehme R, Hartelt K, Petney T, Bücher T, Kimmig P, Taraschewski H. European hedgehogs as hosts for Borrelia spp., Germany. Emerg Infect Dis. 2007; 13(6):952-953.

30. Şen E. Lyme hastalığının epidemiyolojisi. Türk Mikrobiol Cem Derg. 2006; 36(1):55-66.

31. Bayındır Y: Boreliyoz. Zoonozlar: Hayvandan İnsanlara Bulaşan Enfeksiyonlar. M. Doğanay, N. Altıntaş (eds). İzmir. Bilimsel Tıp Yayınevi. 2009; 71-83.

32. Guner ES, Hashimoto N, Takada N, Kaneda K, Imai Y, Masuzawa T. First isolation and characterization of Borrelia burgdorferi sensu lato strains from Ixodes ricinus ticks in Turkey. J Med Microbiol. 2003; 52:807-813.

33. Gargılı A, Midilli K, Ergin S, Şengöz G, Derviş E, Estrada Pena A. First record of Borrelia spielmani in Turkey. Kafkas Univ Vet Fak Derg. 2009; 16 (Suppl-A):167-169.

34. Guner ES, Watanabe M, Kadosaka T, Polat E, Gargılı A, Gülanber A. Seroepidemiology of Borrelia burgdorferi sensu lato and Anaplasma phagocytophilum in wild mice captured in northern Turkey. Epidemiol Infect. 2005; 133:331-336.