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Endosymbiotic bacteria in ticks in Kırşehir, Central Anatolia

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ABSTRACT

Ticks are parasites and vectors, whose diet is blood and hosts are vertebrates. Therefore, they are a risk factor for both, public health and farm animals. Ticks have a cosmopolitan distribution, and their prevalence and incidence of diseases caused by them are increasing with the effect of various environmental factors such as global warming. The use of endosymbiotic bacteria (EB) to control pests is a promising environment-friendly approach as an alternative to chemical methods. Therefore, it is necessary to elucidate the ticks symbionts to develop potentially alternative strategies for managing their populations. Aim of this study, was to search for EB in ticks obtained from livestock in Kırşehir, Central Anatolia. EB were identified by morphological and molecular methods. Investigation was conducted on most studied EB in *Haemaphysalis* spp. and *Hyalomma* spp. using *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Rickettsia*, *Spiroplasma* and *Wolbachia* specific primers. As a result of the survey, *Rickettsia* was detected in all locations and samples, while *Spiroplasma* was detected only one sampling locality. It was that *Rickettsia* is not species specific and has a wide wide distribution incidence. *Spiroplasma* was found only in *Ha. sulcata*. In contrary *Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia* were not found in the sampled ticks. On the other hand, although the reason could not be explained, PCR products thought to be the result of non-specific binding with *Arsenophonus* primers and sequence data similar to *Coxiella*-like endosymbiont were obtained. This study does not explain the tick-symbiosis relationship, but the findings are considered important for future studies of tick biology and/or tick-borne diseases.

Key words: endosymbiotic bacteria; *Rickettsia*; *Spiroplasma*; tick

Introduction

Ticks, are worldwide non species specific ectoparasites of vertebrates. As blood-feeding arthropods, ticks threaten human health and due to their negative effects on the livestock cause economic losses. They are vectors of diseases such as Crimean-Congo haemorrhagic fever, Q fever, Lyme disease, Rocky Mountain spotted fever, babesiosis, and Colorado tick fever. Recent studies have reported increases in the incidence of tick-borne diseases and in tick population density (YÜCESAN et al., 2019), and this is a global problem (JONGEJAN and UILENBERG, 2004). Also, perhaps most importantly, global climate change and warming trend cause a northward (BOUCHARD et al., 2019) and upward range expansion (SAJID et al., 2017) in

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tick distribution. On the other hand, commercial activities (international trade routes) and wildlife (the migration route of migratory birds) also have an impact on tick spread. So that, due to its geographical location, Turkey is a corridor for the intercontinental transfer of ticks (from Africa and the Middle East to Europe, the Caucasus and Central Asia) (INCI et al., 2016).

Geography, environmental factors, climate and life cycle are effective in shaping the microbiome of the tick (ZHANG et al., 2019a; POLLET et al., 2020). Bacterial diversity in ticks is explained by bacterial gains through environmental contaminations and/or infections between feeding meals during their developmental period (GOMARD et al., 2021). However, there are also parasitoid-induced bacterial benefits in ticks (TIJSSE-KLASEN et al., 2011). In this context, some symbionts play a role in tick development and nutrition, while others are effective in the survival and transmission of disease-causing pathogens (DURON et al., 2017; ZHANG et al., 2019b; BRINKMANN et al., 2019; GOMARD et al., 2021). Microbiota composition in a tick species differ from region to region due to climatic and niche differences (POLLET et al., 2020). Therefore, complex and dynamic symbiotic flora in ticks has been investigated.

Symbionts in arthropods, especially some maternally inherited bacteria, are notable for their biological roles as well as their manipulatory impact on tick reproduction. Therefore, interest in endosymbiotic bacteria (EB) (*Arsenophonus*, *Cardinium*, *Hamiltonella*, *Rickettsia*, *Spiroplasma* and *Wolbachia*), which can be transferred horizontally and vertically, has increased in recent years (HONG et al., 2002; ZHANG and LIU, 2019a; KHOO et al., 2020). In arthropods, the effects of EB on the phenotype are generally observed in four ways; male-killing (WERREN et al., 1994), feminization (ROUSSET et al., 1992), parthenogenesis (ZCHORI-FEIN et al., 2001), and cytoplasmic incompatibility (O'NEILL et al., 1992). These bacteria, which also play a role in gene transfer, are examined for the determination and use of effective, environmentally friendly and alternative control strategies such as sterile insect technique due to their symbiotic relationships with their hosts (HONG et al., 2002; ZHANG and LIU, 2019b; KHOO et al., 2020). Therefore, screening, detection and diagnosis of these bacteria in different regions, especially in arthropod pests, are the one most interesting topics in recent years (DURON et al., 2014; ZHANG et al., 2018; ZHANG and LIU, 2019a; LIU et al., 2019; KHOO et al., 2020; TORRES et al., 2020; IPEKDAL and KAYA, 2020). EB is promising for pest control, but more data on the presence of these bacteria in natural populations are needed for pest management. Thus, the aim of this study, was to investigate EB bacteria in ticks obtained from livestock in Kırşehir, which is located in the central of Turkey.

Materials and methods

Tick Sampling. The tick samples examined in this study were collected from five different cattle and sheep barns (n = 53) in December 2020 in Kırşehir, Turkey (Figure 1, Table 1). There were no precautions taken against ticks in the sampling localities where traditional livestock methods were applied. The ticks were disinfected in 70% alcohol for 30 seconds, rinsed with sterilized distilled water at the sampling locality and stored at -20 °C in alcohol. Sampled ticks were identified under a dissecting microscope according to NOSEK and SIXL (1972). Before DNA isolation, samples were disinfected with 70% ethanol for 30 seconds and then rinsed with sterilized distilled water.

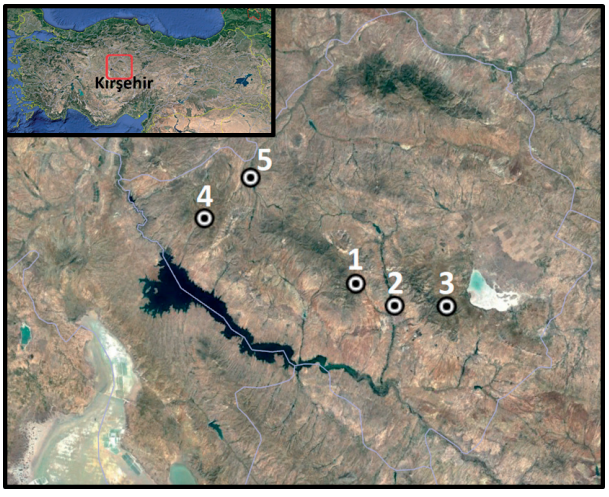

Fig. 1. Tick sampling localities in Kırşehir, Turkey.

Table 1. Sampling localities and coordinates, hosts, sex (N: number; F: female; M: male), and tick genera

No	Locality	Coordinates	Host	N/Sex	Genus
1	Karıncalı, Central	39.132260 34.312030	Cow	4/F + 2/M	*Haemaphysalis* spp.
2	Kervansaray, Central	39.101986 34.919420	Sheep	4/F + 3/M	*Hyalomma* spp.
3	Dalakçı, Mucur	39.102175 34.181322	Cow	4/F + 1/M	*Hyalomma* spp.
				4/F + 1/M	*Haemaphysalis* spp.
4	Karkınmeşe, Kaman	39.205558 33.375834	Sheep	13/F + 3/M	*Haemaphysalis* spp.
5	Yukarı Çiftlik, Akpınar	39.261577 33.452601	Sheep	9/F + 5/M	*Hyalomma* spp.

DNA Extraction and Diagnostic PCR. In order to confirm the morphological identification of the thick species and screening the EB, DNA extraction was conducted from all of the collected tick individuals by using the CTAB method (DOYLE and DOYLE, 1990). For molecular identification of the tick species, LCO1490-F (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198-R (5'-TAAACTTCAGGGTGACCA AAAAATCA-3') primers that amplify a 710 bp region of mitochondrial COI gene were used (FOLMER et al., 1994). To determine the presence of EB, PCR amplifications were performed using 0.5 µl mixtures each containing 10 mM dNTP, 1 µM of each primer, 0.1 U Taq DNA polymerase, 1 x PCR buffer, and 1 µl DNA. Specific primers for *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Rickettsia*, *Spiroplasma* and *Wolbachia*, which are the most commonly studied EB genera, were used for amplification (Table 2). EB screening was performed by using electrophoresis of the PCR products (5 µl) from each tick individual in 1% agarose gel with negative and corresponding positive controls. After the running and staining with Safe-Green (ABM, G108-G), gels were scanned and photographed on a UV Transilluminator (ThermoScientific). Samples with bands at the same position as the positive control were considered positive for the presence of the corresponding EB.

Table 2. Specific primers used for screening the endosymbionts *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Rickettsia*, *Spiroplasma*, and *Wolbachia*, and their characteristics

Primer	Sequence (5'-3')	Target genus and gene region	PCR product (bp)	Annealing (°C)	Reference
Ars-F	GGGTTGTAAAGTACTTTCAGTCGT	*Arsenophonus* 16S rRNA	800	52	DURON et al. (2008)
Ars-R2	GTAGCCCTRCTCGTAAGGGCC				
Ars-R3	CCTYTATCTCTAAAGGMTTCGCTGGATG		600	54	
Clo-F	GCGGTGTAAAATGAGCGTG	*Cardinium* 16S rRNA	466	54	WEEKS et al. (2003)
Clo-R	ACCTMTTCTTAACTCAAGCCT				
Ham-F	TGAGTAAAGTCTGGAATCTGG	*Hamiltonella* 16S rRNA	730	54	ZCHORI-FEIN and BROWN (2002)
Ham-R	AGTTCAAGACCGCAACCTC				
Rb-F	GCTCAGAACGAACGCTATC	*Rickettsia* 23S rRNA	900	58	GOTTLIEB et al. (2006)
Rb-R	GAAGGAAAGCATCTCTGC				
63-F	GCCTAATACATGCAAGTCGAAC	*Spiroplasma* 16S rRNA	450	55	FUKATSU and NIKOH (2000), MATEOS et al. (2006)
TK55-R	TAGCCGTGGCTTTCTGGTAA				
Wspec-F	YATACCTATTCGAAGGGATAG	*Wolbachia* 16S rRNA	430	53	WERREN and WINDSOR (2000)
Wspec-R	AGCTTCGAGTGAAACCAATTC				

Sequencing and Sequence Analysis. One DNA sample for each morphologically identified tick group was sequenced from at least one female individual from each locality. As for EB, at least one DNA sample among those which gave positive band was sequenced. Reverse and forward sequencing was performed by Macrogen Inc., Netherlands. Taxonomic deductions made in this study were obtained by using the Clustal W 2.0 algorithm (THOMPSON et al., 1994) in BioEdit (HALL, 1999). Taxonomic descriptions for both bacteria and ticks were verified using NCBI databases using consensus sequences through BLAST.

Results

Identified Tick Species. A total of 53 individuals (male:female ratio = 16:37) belonging to the genera *Haemaphysalis* spp. (27 individuals) and *Hyalomma* spp. (26 individuals) were found from five sampling localities according to morphological and molecular identification. Tick species determined according to molecular identification were as follows: *Ha. parva*, *Ha. sulcata*, *H. impeltatum* and *H. marginatum* (Table 3).

Table 3. Molecular identification of the tick species collected from Kırşehir, Turkey and GenBank accession numbers of the corresponding matches with the best E-values

No	Locality	Tick Species (n)	GenBank **Accession Number**	E-value
1	Karıncalı, Central	*Ha. parva* (6)	MT230039.1	0.0
2	Kervansaray, Central	*H. impeltatum* (7)	KT989630.1	0.0
3	Dalakçı, Mucur	*Ha. sulcata* (5)	MH532301.1	0.0
		H. marginatum (5)	MN853165.1	0.0
4	Karkınmeşe, Kaman	*Ha. sulcata* (16)	MH532299.1	0.0
5	Yukarı Çiftlik, Akpınar	*H. impeltatum* (14)	KT989630.1	0.0

Endosymbionts detected in tick samples. *Rickettsia* was the only EB found in all of the tick samples from all sampling localities (Figure 2a, Table 4). There was also no relationship between *Rickettsia* occurence and host (cow or sheep) and the sex of the ticks. *Spiroplasma* was detected only in *Ha. sulcata* sampled from Karkınmeşe, Kaman (Figure 2b, Table 4). Thus, this individuald identified as *Ha. sulcata*, was found to have a cooccurence of *Rickettsia* and *Spiroplasma*. The frequency of *Spiroplasma* in Karkınmeşe samples (number of EB positive individuals of the genus / total number of individuals of the genus) was 0.6. *Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia* could not be detected in the tick samples screened (Table 4). Although *Arsenophonus* was not detected in any sample, the sequence of PCR products (Figure 2c) obtained by using *Ars*-F/R2 primers, which are specific to *Arsenophonus*, BLAST-matched with *Coxiella* sp., which is a human Q Fever -disease agent transmitted by ticks (e.g., MH645185.1; Query Cover: 100%, e-value: 0.0, Per. Ident.: 100%). On the other hand, Ars-F/R3, the other *Arsenophonus*-specific primer used, did not obtain PCR product (Figure 2c).

Discussion

Maternally inherited endosymbiotic bacteria were screened in 53 tick samples collected from barns in Kırşehir (Central Anatolia). As a result of the scans performed using specific primers, *Ricketsia* was detected in all tick samples, while *Spiroplasma* was detected in only one location. However, *Arsenophonus*, *Cardinium* and *Hamiltonella* were not detected in the surveyed ticks. In addition, as a result of the investigation, PCR products of the tick parasitoid and human pathogen *Coxiella* were obtained, interestingly, with *Arsenophonus* primers pair Ars-F/R2 (which is thought to be the result of non-specific pairing).

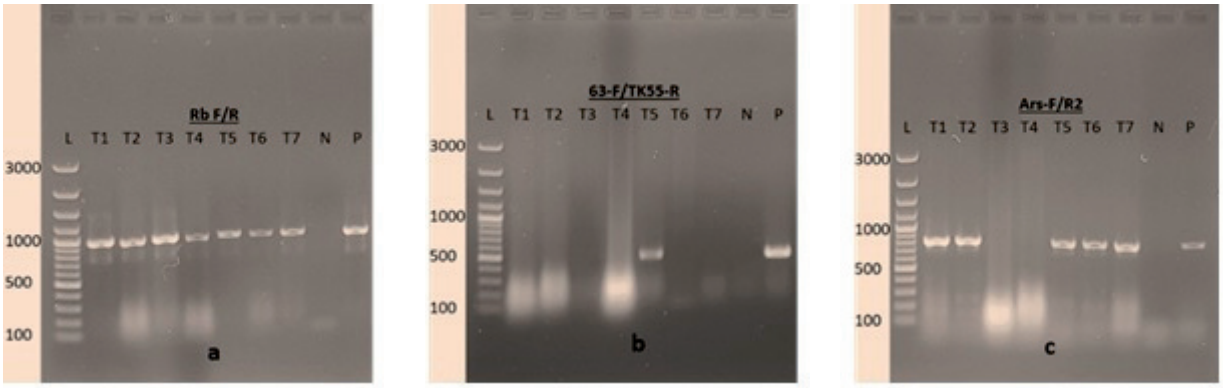

Fig 2. Gel electorphoresis images of the PCR products belonging to (a) *Rickettsia* (Rb-F/R), (b) *Spiroplasma* (63-F/TK55-R), (c) *Arsenophonus* (Ars-F/R2) primers (L: ladder; N: negative control; P: positive control).

Table 4. Tick samples and endosymbionts secrenning results (EB presence ratio = EB-positive individuals / total number of individuals scanned; A: *Arsenophonus*, C: *Cardinium*, H: *Hamiltonella*, R: *Rickettsia*, S: *Spiroplasma*, W: *Wolbachia*)

Locality		Tick Species		EB (presence ratio)					
No	Location	Code	(EB presence ratio)	A	C	H	R	S	W
1	Karıncalı, Central	T1	*Haemaphysalis spp.* (1.0)	-	-	-	+ (1.0)	-	-
2	Kervansaray, Central	T2	*Hyalomma spp.* (1.0)	-	-	-	+ (1.0)	-	-
3	Dalakçı, Mucur	T3	*Haemaphysalis* spp. (1.0)	-	-	-	+ (1.0)	-	-
		T4	*Hyalomma* spp. (1.0)	-	-	-	+ (1.0)	-	-
4	Karkınmeşe, Kaman	T5	*Haemaphysalis* spp. (1.0)	-	-	-	+ (1.0)	+ (0.6)	-
5	Yukarı Çiftlik, Akpınar	T6	*Haemaphysalis* spp. (1.0)	-	-	-	+ (1.0)	-	-

Identification of ticks collected from barns where stockbreeding was done with traditional methods was made using morphological and molecular methods. It was found in the sampling region that *Haemaphysalis* spp. (27 individuals) and *Hyalomma* spp. (26 individuals) has a prevalent incidence. The tick species were identified as *Ha. parva* (6), *Ha. sulcata* (21), *H. impeltatum* (21) and *H. marginatum* (5). In previous studies, 51 tick species have been reported in mammals, reptiles and birds in Turkey (KESKIN and ERCIYAS-YAVUZ, 2019; BURSALI et al., 2020). The most common and most important tick genera in Anatolia are *Haemaphysalis*, *Hyalomma*, *Rhipicephalus* (*Boophilus*), *Dermacentor* and *Argas* (AYDIN and BAKIRCI, 2007). *Haemaphysalis* and *Hyalomma* genera, which are hosts of pathogens that cause diseases such as babesiosis, theileriosis, anaplasmosis and Crimean-Congo haemorrhagic fever, spread in Central Anatolia (AYDIN and BAKIRCI, 2007; MAMAK et al., 2006; ORKUN, 2018; YÜCESAN et al., 2019). However, *Ha. parva* is the most common species (MAMAK et al., 2006), but *Hyalomma* species is also reported to have an increasing trend (YÜCESAN et al., 2019). Although the tick species detected in this study are similar to previous studies, more studies on tick diversity are needed.

Six EB from four species (*Ha. parva*, *Ha. sulcata*, *H. impeltatum* and *H. marginatum*) were investigated with endosymbiotic bacteria-specific primers. As a result of the screening, *Rickettsia* (1.0)

was detected in all sampling localities and ticks. The results obtained showed that the prevalence of *Rickettsia* in ticks in the sampling region was high, regular and not species-specific. The presence of *Rickettsia* in ticks has been previously investigated in terms of antigenic characters in Turkey and this virulence factor has been determined (ORKUN et al., 2014; BRINKMANN et al., 2019). However, *Rickettsia* species are considered in four groups in arthropods according to their biological, phylogenetic and virulence characteristics: spotted fever, typhus, transitional and ancestral groups (GILLESPIE et al., 2007). Among them, *R. bellii* (GILLESPIE et al., 2007), which is in the ancestral group, is expressed as a male killing reproductive manipulator, especially in arthropods (WERREN et al., 1994). In addition, although the exact role of *Rickettsia* in ticks is not known (DURON et al., 2017), it is one of the dominant symbiont genera (WANG et al., 2018). Although the results obtained in this study are similar to previous studies in terms of the presence of *Rickettsia* in ticks, they are insufficient to explain the effects of *Rickettsia* detected in ticks. On the other hand, while *Spiroplasma* was not detected in Mucur Dakakçı village *Ha. sulcata* samples, it was detected in *Ha. sulcata* samples from Karkınmeşe-Kaman. This is the first record of *Spiroplasma* in *Ha. sulcata*. Additionally, double infection (*Rickettsia* and *Spiroplasma*) in *Ha. sulcata* was detected in this study for the first time. *Spiroplasma* has been previously searched for but not detected in *Haemaphysalis* and *Hyalomma* genera (DURON et al., 2017). In contrary, it was detected in *Ixodes* spp., *Rhipicephalus* spp., and *Dermacentor marginatus* (DURON et al., 2017; ZHANG et al., 2018). Both endosymbionts cause reproductive manipulation (male killing) (PERLMAN et al., 2006; MONTENEGRO et al., 2006), play a role in digestion, defense (MONTENEGRO et al., 2006), genetic diversity and evolution (LIU et al., 2019) of their hosts. Therefore, there is a need for studies to be carried out with a data set to be obtained from more samples in order to determine the dynamic symbiotic composition of ticks.

Hamiltonella, *Wolbachia*, *Cardinium* and *Arsenophonus* infections were not detected in *Ha. parva*, *Ha. sulcata*, *H. impeltatum* and *H. marginatum*. This study is the first known study on *Hamiltonella* screening in ticks. However, *Wolbachia*, *Cardinium* and *Arsenophonus* endosymbionts have been previously searched for in ticks. Accordingly, *Wolbachia* infection was detected in *Haemaphysalis* spp. (*Ha. bispinosa, Ha. wellingtoni*) (KHOO et al., 2016) and *Ixodes* spp. (TIJSSE-KLASEN et al., 2011; ZHANG et al., 2011; DURON et al., 2017); *Cardinium* in *Ornithodoros* spp., *Ixodes* spp. and *Rhipicephalus* spp. (DURON et al., 2017); *Arsenophonus* in *Ha. longicornis* (LIU et al., 2013) and *Dermacentor* spp. (DURON et al., 2017). On the other hand, it has been reported that *Wolbachia*, *Cardinium* and *Arsenophonus* are not found in *Ha. parva*, *H. impeltatum* and *H. marginatum* (DURON et al., 2017. The results obtained here are similar to the previous results. However, there is a need for more comprehensive studies in ticks with dynamic and diverse symbiotic compositions. Such that, PCR products of *Coxiella*-like bacteria were obtained, which were interpreted as nonspecific binding results in *Arsenophonus* searches.

Arsenophonus is known for its male killing phenomenon in arthropods (SKINNER, 1985) and has also been studied in ticks (DURON et al., 2008; MEDIANNIKOV et al., 2012). *Arsenophonus* screening in ticks was previously performed with primer pair 554F/NC-Arsen16S-R in *Dermacentor andersoni* (DERGOUSOFF and CHILTON, 2010) and primer pair rpoB/ftsY in *Ixodes ricinus* (MEDIANNIKOV et al., 2012). In this study, *Arsenophonus* was screened with ArsF/ArsR2-R3 primers (Table 2). While 800 bp PCR product and clean sequences were obtained in all ticks (except Dalakçı Village, Mucur samples) with ArsF/R2 primer pair, no PCR product was obtained with ArsF/ArsR3 primer. As a result of the BLAST analysis of the consensus sequences formed with the sequence data of the PCR products obtained from the ArsF/ArsR2 primer pair, 100% similarity with *Coxiella*-like bacteria, one of the pathogenic parasites in ticks (MH645185.1). The reason for this situation has been interpreted as the result of non-specific binding. The study acknowledges the uncertainties associated with conventional PCR detection (such

as high false-positive detection rates). Taking this into account, the scans were meticulously carried out. However, interestingly, the aforementioned PCR product was not obtained in the samples taken from the Dalakçı Village, Mucur location (Table 4). However, according to the BLAST analysis results, even if there is no specific binding, it can be interpreted as an indication of the presence of *Coxiella*-like bacteria in the studied samples, but to be sure od that it needs confirmation. In fact, the presence of *Coxiella* in ticks has recently been reported in Turkey (BRINKMANN et al., 2019). Co-infection of *Arsenophonus* and *Coxiella* in ticks has also been reported (LIU et al., 2013; GOMARD et al., 2021). Additionally, it is reported that both bacteria are maternally inherited in arthropods (LIU et al., 2013; DURON et al., 2014; DURON et al., 2016), interact (GOMARD et al., 2021) and are the closest taxa in terms of sequence similarity (LIU et al., 2013). Especially *Coxiella*, evolution of which is poorly known, is a trending research topic (LIU et al., 2013; DURON et al., 2015; GOMARD et al., 2021). It is obvious that there is a need for larger-scale studies including *Coxiella* in ticks and data to be obtained in this context.

Conclusions

As a result, endosymbionts were investigated in *Ha. parva*, *Ha. sulcata*, *H. impeltatum* and *H. marginatum* ticks, and *Rickettsia* was found to have a widespread prevalence. However, to our knowing *Spiroplasma* and *Rickettsia* co-infection were detected for the first time so far in *Ha. sulcata* is known. *Arsenophonus*, *Cardinium*, *Hamiltonella* and *Wolbachia* infections were not detected in any of the studied tick specimens. On the other hand, findings of *Coxiella*-like tick endosymbionts were obtained by chance, and interestingly, with primers specific for *Arsenophonus*. It is thought that the data obtained are important for studies to be done both to illuminate the dynamic and complex symbiotic relationship in tick biology and to develop effective and alternative control methods against tick-borne diseases and even possible epidemics. However, the findings not are of sufficient value to explain the relationship between ticks and symbionts, and more studies are needed.

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SAŽETAK

Krpelji su paraziti i vektori koji se hrane krvlju, a domaćini su im kralježnjaci. Zbog toga su rizičan čimbenik i za javno zdravlje i za životinje u farmskom uzgoju. Krpelji se nalaze širom svijeta, a prevalencija i incidencija bolesti koje uzrokuju u porastu su zbog utjecaja različitih čimbenika okoliša, poput globalnog zatopljenja. Upotreba endosimbiotskih bakterija (EB) u svrhu kontrole štetočina obećavajuća je ekološki prihvatljiva alternativa kemijskim metodama. Kako bi se razvile potencijalne alternativne strategije za upravljanje populacijama krpelja, potrebno je razjasniti njihove simbionte. Cilj je ovog istraživanja bio izdvojiti EB iz krpelja pronađenih u stoke u Kırşehir pokrajini Srednje Anadolije. Endosimbiotske bakterije identificirane su morfološkim i molekularnim metodama. Istraživanje je provedeno na najčešće promatranim endosimbiotskim bakterijama u vrstama *Haemaphysalis* spp. i *Hyalomma* spp. upotrebom primera specifičnih za *Arsenophonus*, *Cardinium*, hamiltonele, rikecije, spiroplazme i volbahije. Kao rezultat istraživanja rikecija je pronađena na svim mjestima i u svim uzorcima, a spiroplazma na samo jednom mjestu uzorkovanja. Pokazalo se da rikecija nije specifična za vrste i da je široko rasprostranjena. Spiroplazma je pronađena samo u vrsti *Ha. sulcata*. Nasuprot tome, *Arsenophonus*, *Cardinium*, hamiltonela i volbahija nisu pronađeni u uzorcima krpelja. S druge strane, premda razlog nije otkriven, dobiveni su produkti PCR-a za koje se smatralo da su rezultat nespecifičnog vezanja s primerima *Arsenophonus* i sekvencom koja se podudara s *Coxiella*-sličnom endosimbiontom. Ovo istraživanje ne objašnjava odnos između krpelja i simbionata, no rezultati se smatraju važnima za buduća istraživanja biologije krpelja i bolesti koje oni prenose.

Ključne riječi: endosimbiotske bakterije; rikecija; spiroplazma; krpelj