

Molecular detection of *Francisella tularensis* and *Rickettsia* spp. in ticks collected on animals in the pasture, Edirne

EDA KIRILMAZ

eddakirilmaz@hotmail.com

Çankırı State Hospital

DENİZ SUMNULU

Technology Research Development Application and Research Center

Selahattin Çağatay KONCE

Trakyalılar Veterinary Clinic, Edirne, Türkiye

Levent AYDIN

Bursa Uludağ University, Faculty of Veterinary Medicine

HÜSEYİN GUDUCUOĞLU

Department of Medical Microbiology, Faculty of Medicine, Trakya University, Edirne

İSMAIL DAVARCI

Department of Medical Microbiology, Faculty of Medicine, Trakya University, Edirne

Research Article

Keywords: *Francisella tularensis*, *Rickettsia* spp., tick-borne diseases, ticks, vector ecology, zoonoses

Posted Date: August 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10403371/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background:

Ticks are important vectors of numerous zoonotic pathogens affecting both human and animal health. *Rickettsia* species are widely distributed tick-borne bacteria, whereas *Francisella tularensis* is a highly infectious zoonotic agent with focal ecological distribution. The Thrace region of Türkiye represents an epidemiological transition zone due to its geographical location and ecological characteristics.

Methods:

Between June and September 2023, ticks were collected from grazing cattle and sheep in the Keşan and İpsala districts of Edirne Province. A total of 540 ticks were morphologically identified and pooled in groups of ten according to species and collection area, resulting in 54 DNA pools. Molecular analyses were performed using polymerase chain reaction (PCR). The *tu14* gene was targeted for the detection of *F. tularensis*, while the *gltA*, *ompA*, and 23S rRNA gene regions were targeted for *Rickettsia* spp. Positive samples were subjected to nucleotide sequencing for species-level identification.

Results:

The collected ticks were identified as *Rhipicephalus sanguineus* (61.9%), *Rhipicephalus annulatus* (29.8%), and *Hyalomma marginatum* (8.3%). No PCR positivity was detected for *F. tularensis*. In contrast, *Rickettsia* spp. DNA was detected in 8 of the 54 pools (14.8%). Sequence analysis identified *Candidatus Rickettsia barbariae* and *Rickettsia massiliae*. Positivity was predominantly observed in *Rhipicephalus* species, indicating the circulation of multiple *Rickettsia* species within shared ecological niches in the region.

Conclusions:

This study demonstrates the molecular presence of tick-borne *Rickettsia* species in the Thrace region of Türkiye and highlights the region as an active ecological area for rickettsial circulation. Although *F. tularensis* was not detected, the continued risk of tularemia through environmental and waterborne transmission should not be overlooked. These findings emphasize the importance of integrated, One Health-based molecular surveillance programs to assess and mitigate zoonotic risks in Thrace and neighboring Balkan regions.

1. Introduction

Ticks are important vectors involved in the transmission of numerous bacterial, viral, and parasitic agents affecting both humans and animals. Climate change, environmental factors, and the disruption of ecological balance have contributed to increases in tick populations and to the expanded spread of tick-borne zoonotic diseases [1]. Owing to its geographical location, climatic diversity, and livestock-based economic structure, Türkiye is among the regions where ticks and tick-borne pathogens are endemic [2].

Among tick-borne diseases, zoonotic infections caused by *Rickettsia* and *Francisella* species are of particular importance for both veterinary medicine and public health [3]. *Francisella tularensis* is considered a significant biological agent due to its low infectious dose, environmental persistence, and potential for aerosol transmission [4]. Tularemia can be transmitted to humans via ticks, rodents, and contaminated water sources; in Türkiye, waterborne and tick-associated cases have been frequently reported [5, 6].

Rickettsia species are obligate intracellular, Gram-negative bacteria responsible for various clinical syndromes, most notably those belonging to the spotted fever group (SFG). In the Mediterranean basin, *Rickettsia conorii* is the most prevalent species and has been molecularly detected in both human cases and various tick species in Türkiye [7, 8]. In recent years, the identification of additional species such as *R. aeschlimannii*, *R. slovaca*, *R. raoultii*, and *R. hoogstraalii* in Türkiye has indicated that the ecological spectrum of rickettsioses is broader than previously assumed [9, 10].

Advances in molecular methods have enabled more sensitive and reliable identification of tick species and the pathogens they harbor. PCR and sequencing analyses targeting the *gltA*, *ompA*, and *ompB* gene regions are widely used for the identification of *Rickettsia* species, whereas PCR and sequencing of the *tu14* gene are commonly employed for the confirmation of *Francisella* species [7, 9, 11]. Using these approaches, studies conducted in different regions of Türkiye have demonstrated a widespread presence of *Rickettsia* DNA in ticks, while the detection of *Francisella tularensis* has remained limited [2, 12, 13].

The broad host range of rickettsial infections and their association with diverse tick species suggest that ecological and climatic factors play a decisive role in the regional distribution of these diseases [14]. Particularly in transition-zone countries such as Türkiye, determining the diversity and geographic distribution of tick species is of critical importance for anticipating potential zoonotic threats [1].

In this context, the present study aimed to investigate the presence of *Francisella tularensis* and *Rickettsia* spp. in ticks collected from grazing animals in the Keşan and İpsala districts of Edirne Province using molecular methods. The findings are expected to provide valuable insights into regional vector ecology and to support the assessment of potential zoonotic risks.

2. Materials and methods

2.1.Ethical approval

This study was approved by the Scientific Research Ethics Committee of Trakya University Faculty of Medicine (Approval No: TÜTF-GOBAEK 2022/356).

2.2.Study area and sample collection

This study was conducted between June and September 2023 in the Keşan district of Edirne Province (40°51'N, 26°38'E), including the villages of Karahisar, Kılıçköy, Kızkapan, Orhaniye, and Büyükdoğanca, as well as in the İpsala district (40°55'N, 26°22'E), including the villages of Yapıldak, Koyuntepe, and Aliçopehlivan. Ticks were collected from barn animals grazing on pastures in these areas. Both regions are characterized by wetland and rural environments with suitable microclimatic conditions for ticks, where agriculture and livestock farming are widespread in Thrace [1].

Ticks were visually detected during physical examination of grazing large and small ruminants and were removed alive using forceps. For each animal, the number of collected ticks, host species, date of collection, and location were recorded. Samples were placed in tubes containing 96% ethanol and transported to the laboratory under cold-chain conditions. Damaged ticks or those that were recently engorged were excluded from the study.

2.3.Morphological identification of ticks

Following collection, ticks were transported to the Medical Microbiology Laboratory of Trakya University Faculty of Medicine. Morphological identification was performed at the Faculty of Veterinary Medicine, Uludağ University, using a stereomicroscope (Nikon, Tokyo, Japan), and specimens were identified at the species level. Identification was carried out by evaluating morphological characteristics according to the taxonomic keys described by Estrada-Peña and Walker [15, 16].

2.4.DNA extraction

A pooling strategy was applied for DNA extraction from ticks. Ticks were pooled in groups of ten according to species and collection area, resulting in a total of 54 pools. Each tick was bisected using a sterile scalpel; one half was numbered and stored at -20 °C, while the other half was placed into Eppendorf tubes. The tubes were incubated at -150 °C for 5 minutes and subsequently homogenized at maximum speed for 5 minutes using a tissue lyser; this procedure was repeated twice. DNA isolation was performed using the GeneMATRIX Tissue & Bacterial DNA Purification Kit (EurX, Poland) in accordance with the manufacturer’s instructions, and the extracted DNA samples were stored at -20 °C until analysis.

2.5.Molecular analyses

Polymerase chain reaction (PCR) was performed to detect the presence of *Francisella tularensis* and *Rickettsia* spp. in tick samples. The *tul4* gene was targeted for *F. tularensis*, while the *gltA*, *ompA*, and 23S rRNA gene regions were targeted for *Rickettsia* spp. Primer sequences previously described in the literature were used (Table 1) [17-19].

Table 1. Primer sequences used for PCR detection of *Francisella tularensis* and *Rickettsia* spp.

Pathogen	Target Gene	Primer Base Sequences
<i>Rickettsia</i> spp.	23S rRNA	F: AGCTTGCTTTTGGATCATTTGG
		R: TTCCTTGCCTTTTCATACATCTAGT
	ompA	F: ATGGCGAATATTCTCCAAA
		R: AGTGCAGCATTGCTCCCCCT
	gltA	F: GGGGGCTGCTCACGGCGG
		R: ATTGCAAAAAGTACAGTGAACA
<i>Francisella tularensis</i>	tul4	F: ATTACAATGGCAGGCTCCAGA
		R: TGCCCAAGTTTATCGTTCTTC

PCR products were resolved on 2% agarose gels. For agarose gel preparation, 10× TAE buffer (Gibco, USA) was diluted with ultrapure water to obtain a final concentration of 1.5×. A total of 5 g of ultrapure agarose (Invitrogen, USA) was dissolved in 1.5× TAE buffer to a final volume of 100 mL. Before gel solidification, 2.5 µL of SYBR Safe DNA gel stain (Invitrogen, USA) was added. For electrophoresis, 20 µL of each PCR product was mixed with 5 µL of bromophenol blue loading dye (Invitrogen, USA) and loaded onto the gel.

2.6.DNA sequencing analysis and species identification

Nucleotide sequencing analysis was performed on *Rickettsia*-positive samples using the Sanger sequencing method. Prior to sequencing, PCR products were purified using the MAGBIO HighPrep PCR Clean-up System Kit (AC-60005, Canada) according to the manufacturer’s instructions. Forward primers of the *ompA* and *gltA* genes were used for Sanger sequencing. Cycle sequencing reactions were carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Canada) in accordance with the manufacturer's protocol. Sanger sequencing was performed on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, Canada). DNA base sequences were assembled using the BioEdit software, and species-level identification was achieved by comparing the obtained sequences with those available in the NCBI BLAST nucleotide database [20].

3. Results

Between June and September 2023, a total of 540 ticks were collected from animals grazing on pastures in villages of the Keşan and İpsala districts of Edirne Province. The species distribution of the collected ticks was *Rhipicephalus sanguineus* (61.9%), *Rhipicephalus annulatus* (29.8%), and *Hyalomma marginatum* (8.3%).

Ticks were pooled in groups of ten according to species and collection area, resulting in 54 pooled DNA samples.

PCR analyses targeting the *tul4* gene revealed no positivity for *Francisella tularensis*. In contrast, 8 of the 54 pools (14.8%) were positive for *Rickettsia* spp. Four pools were positive for both the *gltA* and *ompA* genes, while four pools showed positivity only for the *ompA* gene. No positivity was detected for the 23S rRNA gene.

Sequencing analysis identified *Rickettsia massiliae* in samples positive only for *ompA*. Pools positive for both *gltA* and *ompA* were classified as *Candidatus Rickettsia barbariae* and *Rickettsia massiliae* based on sequence data (Figure 1).

When the distribution of positive pools by village was examined, the highest number of positive pools was observed in ticks collected from Karahisar village (6/54). Positivity was predominantly detected in *Rhipicephalus sanguineus* and *Rhipicephalus annulatus* species (Table 2)(Figure 2).

Table 2. Distribution of *Rickettsia* spp. gene positivity in ticks collected from villages in Edirne Province according to village, host, and tick species

Villages	Number of ticks collected from cattle	Number of ticks collected from sheep	Rhipicephalus annulatus	Rhipicephalus sanguineus	Hyalomma marginatum	ompA-positive pool	gltA-positive pool	Identified species
Karahisar	62	28	60	30	0	4/54	2/54	C. R. barbariae, R. massiliae
Kılıçköy	3	0	0	3	0	0/54	0/54	-
Kızkapan	120	45	70	95	0	2/54	0/54	R. massiliae
Orhaniye	29	1	0	30	0	1/54	0/54	R. massiliae
Büyükdoganca	27	15	30	12	0	0/54	0/54	-
Koyuntepe	20	10	0	10	20	0/54	0/54	-
Yapıldak	150	20	0	170	0	1/54	2/54	C. R. barbariae, R. massiliae
Aliçopehlivan	6	4	0	0	10	0/54	0/54	-
Total	417	123	160	350	30	8/54	4/54	-

C. R. barbariae: Candidatus Rickettsia barbariae

4. Discussion

In this study, the presence of *Francisella tularensis* and *Rickettsia* spp. was investigated using molecular methods in ticks collected from grazing cattle and sheep in the Keşan and İpsala districts of Edirne Province. While *F. tularensis* was not detected, *Rickettsia* spp. positivity was identified in 14.8% of the samples. Sequence analysis revealed the presence of *Candidatus Rickettsia barbariae* and *Rickettsia massiliae*. The detection of more than one *Rickettsia* species in ticks collected from the same host species suggests the potential co-circulation of multiple rickettsial agents within shared ecological niches in the Thrace region. These findings demonstrate the circulation of tick-borne rickettsial agents in the Thrace region and provide a distinctive picture in terms of the region's ecological, geographical, and epidemiological characteristics.

Thrace represents Türkiye’s European territory and shares borders with Bulgaria to the north and Greece to the west. This geographical position renders the region not only a socioeconomic but also an epidemiological transition zone. Studies conducted in the Balkans and Southeastern Europe

have reported similar *Rickettsia* species, including *R. massiliae*, *R. aeschlimannii*, and *R. slovaca*, in both ticks and human cases [1, 14]. The similarity between the species detected in Thrace and those reported from neighboring regions suggests ecological continuity between Türkiye and Balkan countries and indicates the potential for cross-border dispersal of ticks or reservoir hosts [1, 2, 21]. In particular, the role of migratory birds in the transportation of tick species such as *Hyalomma marginatum* may contribute to the wide geographic dissemination of *Rickettsia* spp. [21].

In Türkiye, most tularemia cases have been reported as waterborne outbreaks [6]. Vector-borne transmission of *F. tularensis* has been primarily associated with tick species linked to rodents [4, 22]. In the present study, the predominance of *Rhipicephalus sanguineus* and *R. annulatus* and the collection of ticks from cattle and sheep may explain the absence of *F. tularensis* detection. Additionally, the low environmental abundance of *F. tularensis* and its focal distribution in nature may result in low detection rates [11]. Nevertheless, previous waterborne tularemia outbreaks in Thrace indicate that the regional reservoir of the pathogen has not been completely eliminated [23, 24]. Therefore, continued surveillance of *F. tularensis* in different hosts (e.g., rodents and hares) and vectors (e.g., *Dermacentor marginatus*) is recommended.

In this study, *Rickettsia* spp. positivity was detected at a rate of 14.8%. Previous studies have demonstrated considerable regional variation, with reported infection rates of 41.3% in Istanbul [25], 10.5% in Yozgat [9] and 27.2% in Ankara [26], while a separate investigation from Çorum documented a prevalence of 31% [27]. In Slovakia, *Rickettsia* spp. positivity rates of 3% and 27% have been reported in ticks collected from sheep and vegetation, respectively, and studies from Southern Europe indicate substantial variability in rickettsial prevalence among tick populations [14, 28, 29]. This variability supports the notion that *Rickettsia* species exhibit high eco-regional distribution.

Among the two species identified in this study, *R. massiliae* has been associated with Mediterranean spotted fever–like clinical manifestations in humans within the Mediterranean basin [30]. *Candidatus R. barbariae* was first described in *Rhipicephalus bursa* ticks in Portugal and has subsequently been reported in Tunisia, Morocco, Spain, and China [2, 31]. The detection of this species in Türkiye provides important molecular evidence for its presence in the country. These findings suggest that the climatic and ecological structure of Thrace shares similarities with Southern European and North African regions in terms of *Rickettsia* species diversity.

The predominance of positivity in *Rhipicephalus sanguineus* and *R. annulatus* supports the role of these tick species as competent vectors for *Rickettsia* spp. [9]. *Rhipicephalus sanguineus* is widely recognized as a major vector of zoonotic *Rickettsia* species due to its close association with domestic dogs and its ability to adapt to human settlements. Indeed, the frequent identification of human pathogens such as *R. conorii* and *R. massiliae* in this species in Iran, Morocco, Palestine, and Tunisia highlights its critical role in zoonotic transmission [14]. Furthermore, the widespread presence of *R. annulatus* in cattle-based farming systems in Türkiye [27] and the association of *R. massiliae* with various tick species linked to animal reservoirs suggest that this pathogen may play a role in animal-to-human transmission. In addition, the high sensitivity of tick seasonal activity to microclimatic conditions such as temperature and humidity, together with the effects of climate change on these parameters, indicates that significant shifts in transmission patterns may occur in the future [32].

The *gltA* and *ompA* gene regions used in this study are widely recognized as molecular targets with high discriminatory power for the identification of *Rickettsia* species. Indeed, these genes are among the most frequently employed markers for species-level confirmation and classification in the literature [10, 33]. While the *gltA* gene serves as a sensitive genus-level marker due to its conservation across *Rickettsia* species, *ompA* allows for species differentiation within the spotted fever group (SFG). The higher rate of *ompA* positivity observed in this study indicates the predominance of SFG members and suggests that the regional *Rickettsia* ecosystem is largely SFG-oriented [9, 33]. The absence of positivity for the 23S rRNA gene may reflect its relatively lower sensitivity.

Rickettsial infections, owing to their zoonotic nature, lie at the intersection of human, animal, and environmental health and thus represent a typical example of the One Health approach [34]. In regions such as Thrace, where agriculture and livestock farming are widespread, farmers, shepherds, and rural communities are among the highest-risk groups for tick exposure due to their continuous contact with natural tick habitats [35]. Moreover, *Rhipicephalus sanguineus*, which has developed strong ecological adaptation to domestic dogs, can feed readily on dogs throughout all life stages [36] and adapt to human living environments, including indoor settings, under favorable conditions [34]. Consequently, this vector plays a critical role in introducing zoonotic *Rickettsia* species—particularly *R. conorii* and *R. massiliae*—into household environments, a relationship that has been well documented in the literature [36]. Given Thrace's proximity to the Balkans, the transboundary nature of zoonotic diseases becomes particularly relevant. Accordingly, the establishment of regional vector surveillance collaborations among Bulgaria, Greece, and Türkiye, the sharing of epidemiological data through joint platforms, and the international coordination of training programs are strongly recommended.

The limitations of this study include the relatively limited sample size, sampling restricted to a single season, and the examination of ticks collected only from specific host species (cattle and sheep). Nevertheless, the molecular identification of pathogens at the species level and the analysis of three different gene regions represent notable strengths of the study. Future research incorporating additional host groups such as rodents, hares, dogs, and birds would contribute to a more comprehensive understanding of *Rickettsia* ecology.

The detection of *Candidatus R. barbariae* and *R. massiliae* in Edirne and its surroundings indicates that the Thrace region constitutes an active ecological area for rickettsial circulation. Although *F. tularensis* was not detected in this study, suggesting a low regional prevalence, the continued risk of environmental and waterborne transmission of tularemia should not be overlooked. In light of these findings, the implementation of multicenter and seasonal molecular surveillance programs in Thrace and neighboring Balkan countries, the integration of tick population monitoring with climate change indicators to generate regional risk maps, the development of targeted educational and preventive programs for individuals

engaged in agriculture and livestock farming, and the establishment of data-sharing and joint reporting systems among institutions working in human, animal, and environmental health are of critical importance. Overall, this study represents one of the first molecular investigations demonstrating the presence of *Rickettsia* species in the Thrace region and contributes to a better understanding of the epidemiology of tick-borne rickettsial infections in Türkiye and the Balkan geography. The data obtained provide a scientific basis for strengthening One Health-based surveillance systems at both national and regional levels.

5. Conclusion

This study demonstrates the circulation of *Rickettsia* species in ticks in the Thrace region, revealing that the region is an active focus for rickettsial infections. The absence of *Francisella tularensis* detection indicates that tularemia should be evaluated within the framework of different transmission routes and ecological dynamics in the region. These findings emphasize the importance of continuous surveillance based on the One Health approach in the region.

Abbreviations

PCR – Polymerase Chain Reaction

DNA – Deoxyribonucleic Acid

rRNA – Ribosomal Ribonucleic Acid

SFG – Spotted Fever Group

NCBI – National Center for Biotechnology Information

BLAST – Basic Local Alignment Search Tool

TAE – Tris–Acetate–EDTA

Declarations

6.1.Ethics approval and consent to participate

Not applicable

6.2.Consent for publication

Not applicable

6.3.Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

6.4.Competing interests

The authors declare that they have no competing interests.

6.5.Funding

This study was supported by the Scientific Research Projects Coordination Unit of Trakya University (Project No: TUBAP-2023/18). The funder had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

6.6.Authors' contributions

E.K, İ.D and H.G designed the study. E.K, İ.D, D.Ş L.A and S.Ç.K collected the tick samples. E.K, L.A, D.Ş and S.Ç.K performed the molecular analyses. E.K, L.A, D.Ş, H.G and S.Ç.K conducted the sequencing and data analysis. E.K, İ.D and H.G drafted the manuscript. All authors read and approved the final manuscript.

6.7.Acknowledgements

Not applicable.

References

1. İnci A, Yıldırım A, Düzlü Ö (2016) The current status of ticks in Turkey: A 100-year period review from 1916 to 2016. *Turkish J Parasitol* 40(3):152–157
2. Ji S, Ceylan O, Ma Z, Galon EM, Zafar I, Li H, Hasegawa Y, Sevinç M, Masatani T, Iguchi A et al (2022) Protozoan and rickettsial pathogens in ticks collected from infested cattle in Turkey. *Pathogens* ;11(5)
3. Kirczuk L, Piotrowski M, Rymaszewska A (2021) Detection of tick-borne pathogens of the genera *Rickettsia*, *Anaplasma* and *Francisella* in *Ixodes ricinus* ticks in Pomerania (Poland). *Pathogens* 10(7):901
4. Kılıç S (2006) Biyolojik silah olarak bakteriler: Kategori A ajanlar. *Turkish J Hygiene Experimental Biology* 63(1):21–46
5. İnci A, Yıldırım A, Düzlü O, Doğanay M, Aksoy S (2016) Tick-borne diseases in Turkey: A review based on One Health perspective. *PLoS Negl Trop Dis* 10(12):e0005021
6. Kutlu M, Ergin Ç, Karadenizli A, Sayın Kutlu S (2021) An outbreak of tularemia in southwestern Turkey. *J Infect Developing Ctries* 15(6):812–817
7. Çelebi B, Yeşilyurt M, Kılıç S (2018) A *Rickettsia* case caused by *Rickettsia conorii*. *Mikrobiyoloji bülteni* 52(4):431–438
8. Yılmaz E, Akalın H, Mısıktık R, Heper Y, Engin A, Kılıçaslan E, Öztüfekçi A, Sevgican E, Helvacı S, Töre O (2010) Mediterranean spotted fever: Retrospective evaluation of 16 cases. *Trakya Univ Fac Med J* 27(2):167–171
9. Keskin A, Bursalı A, Keskin A, Tekin S (2016) Molecular detection of spotted fever group rickettsiae in ticks removed from humans in Turkey. *Ticks Tick-borne Dis* 7(5):951–953
10. Emiroğlu M, Çelebi B, Alkan G, Yılmaz Y (2021) The first human case of *Rickettsia slovaca* from Turkey. *Ticks Tick-borne Dis* 12(5):101755
11. Tularemia Sjöstedt A (2007) History, epidemiology, pathogen physiology, and clinical manifestations. *Ann N Y Acad Sci* 1105(1):1–29
12. Güneş T, Ataş M (2020) The prevalence of tick-borne pathogens in ticks collected from the northernmost province (Sinop) of Turkey. *Vector-Borne Zoonotic Dis* 20(3):171–176
13. Demir S, Erkunt Alak S, Köseoğlu AE, Ün C, Nalçacı M, Can H (2020) Molecular investigation of *Rickettsia* spp. and *Francisella tularensis* in ticks from three provinces of Turkey. *Exp Appl Acarol* 81(2):239–253
14. Seidi S, Omidi AH, Esmaili S (2024) Distribution of different *Rickettsia* species in countries of the WHO Eastern Mediterranean Region (WHO-EMRO): An overview. *Travel Med Infect Dis* 58:102695
15. Estrada-Peña A, Bouattour A, Camicas J, Walker A (2004) Ticks of veterinary and medical importance: The Mediterranean basin. University of Zaragoza, Zaragoza
16. Walker JB, Keirans JE, Horak IG (2000) The genus *Rhipicephalus* (Acari, Ixodidae): A guide to the brown ticks of the world. Cambridge University Press, Cambridge
17. Karatuna O, Çelebi B, Can S, Akyar I, Kılıç S (2016) The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry in the identification of *Francisella tularensis*. *Bosnian J Basic Med Sci* 16(2):132–138
18. Jado I, Escudero R, Gil H, Jiménez-Alonso MI, Sousa R, García-Pérez AL, Rodríguez-Vargas M, Lobo B, Anda P (2006) Molecular method for identification of *Rickettsia* species in clinical and environmental samples. *J Clin Microbiol* 44(12):4572–4576
19. Hirunkanokpun S, Ahantarig A, Baimai V, Pramual P, Rakthong P, Trinachartvanit W (2025) Two novel *Rickettsiae* and co-detections of bacteria and protozoa in *Amblyomma* ticks of reptiles from Thailand. *BMC Microbiol* 25(1):463
20. BLAST: Basic Local Alignment Search Tool [Internet]. Available from: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
21. Wallménius K, Barboutis C, Fransson T, Jaenson TG, Lindgren PE, Nyström F, Olsen B, Salaneck E, Nilsson K (2014) Spotted fever *Rickettsia* species in *Hyalomma* and *Ixodes* ticks infesting migratory birds in the European Mediterranean area. *Parasites Vectors* 7:318
22. Telford SR III, Goethert HK (2020) Ecology of *Francisella tularensis*. *Ann Rev Entomol* 65:351–372
23. Akalın H, Helvacı S, Gedikoğlu S (2009) Re-emergence of tularemia in Turkey. *Int J Infect Dis* 13(5):547–551
24. Gürçan S (2014) Epidemiology of tularemia. *Balkan Med J* 31(1):3–10
25. Gargili A, Palomar AM, Midilli K, Portillo A, Kar S, Oteo JA (2012) *Rickettsia* species in ticks removed from humans in Istanbul, Turkey. *Vector-Borne Zoonotic Dis* 12(11):938–941
26. Orkun Ö, Karaer Z, Çakmak A, Nalbantoğlu S (2014) Identification of tick-borne pathogens in ticks feeding on humans in Turkey. *PLoS Negl Trop Dis* 8(8):e3067
27. Karasartova D, Güreşer AS, Gökçe T, Çelebi B, Yapar D, Keskin A, Çelik S, Ece Y, Erenler AK, Usluca S et al (2018) Bacterial and protozoal pathogens found in ticks collected from humans in Çorum Province of Turkey. *PLoS Negl Trop Dis* 12(4):e0006395
28. Lledó L, Giménez-Pardo C (2021) Preliminary evidence of *Rickettsia slovaca* and *Rickettsia conorii* infection in sheep, dogs and deer from northern Spain. *Pathogens* ;10(7)
29. Guccione C, Colomba C, Iaria C, Cascio A (2023) *Rickettsiales* in the WHO European Region: An update from a One Health perspective. *Parasites Vectors* 16(1):41
30. Eldin C, Virgili G, Attard L, Edouard S, Viale P, Raoult D et al (2018) *Rickettsia massiliae* infection after a tick bite on the eyelid. *Travel Med Infect Dis* 26:66–68

31. de Sousa R, Barata C, Vitorino L, Santos-Silva M, Carrapato C, Torgal J, Walker D, Bacellar F (2006) Rickettsia sibirica isolation from a patient and detection in ticks, Portugal. Emerg Infect Dis 12(7):1103–1108
32. Medlock JM, Hansford KM, Bormane A, Derdakova M, Estrada-Peña A, George JC, Golovljova I, Jaenson TG, Jensen JK, Jensen PM et al (2013) Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. Parasites Vectors 6:1
33. Fournier PE, Dumler JS, Greub G, Zhang J, Wu Y, Raoult D (2003) Gene sequence-based criteria for identification of new Rickettsia isolates. J Clin Microbiol 41(12):5456–5465
34. Parola P, Raoult D (2001) Ticks and tick-borne bacterial diseases in humans: An emerging infectious threat. Clin Infect Dis 32(6):897–928
35. Estrada-Peña A, de la Fuente J (2014) The ecology of ticks and epidemiology of tick-borne viral diseases. Antiviral Res 108:104–128
36. Dantas-Torres F (2008) The brown dog tick, Rhipicephalus sanguineus: From taxonomy to control. Vet Parasitol 152(3–4):173–185

Figures

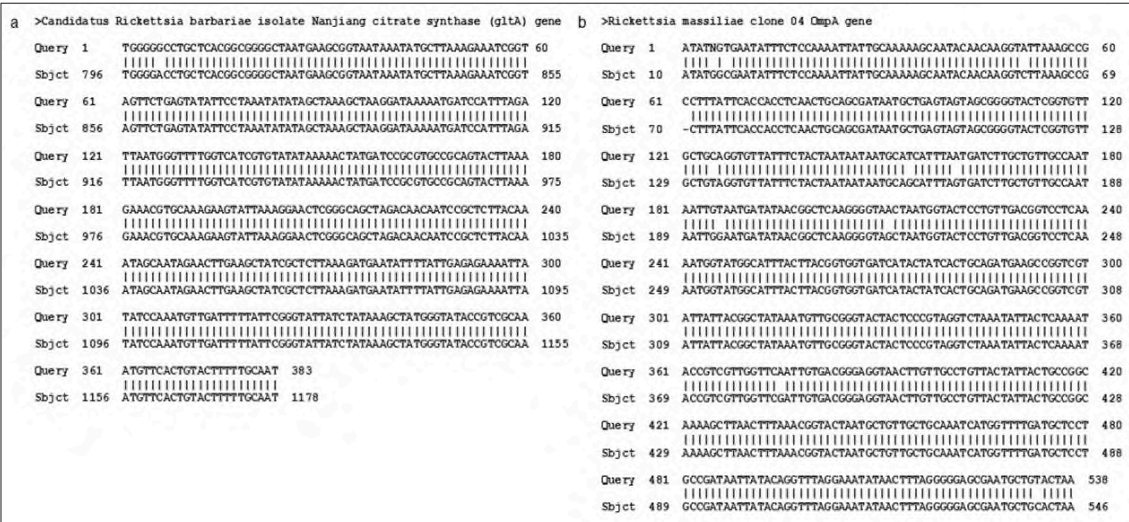


Figure 1. NCBI BLAST nucleotide sequence alignment of PCR products obtained from ticks showing (a) the 796–1178 region (99% identity) of the *gltA* gene of *Candidatus Rickettsia barbariae* and (b) the 10–546 region (98% identity) of the *ompA* gene of *Rickettsia massiliae*

Figure 1

See image above for figure legend

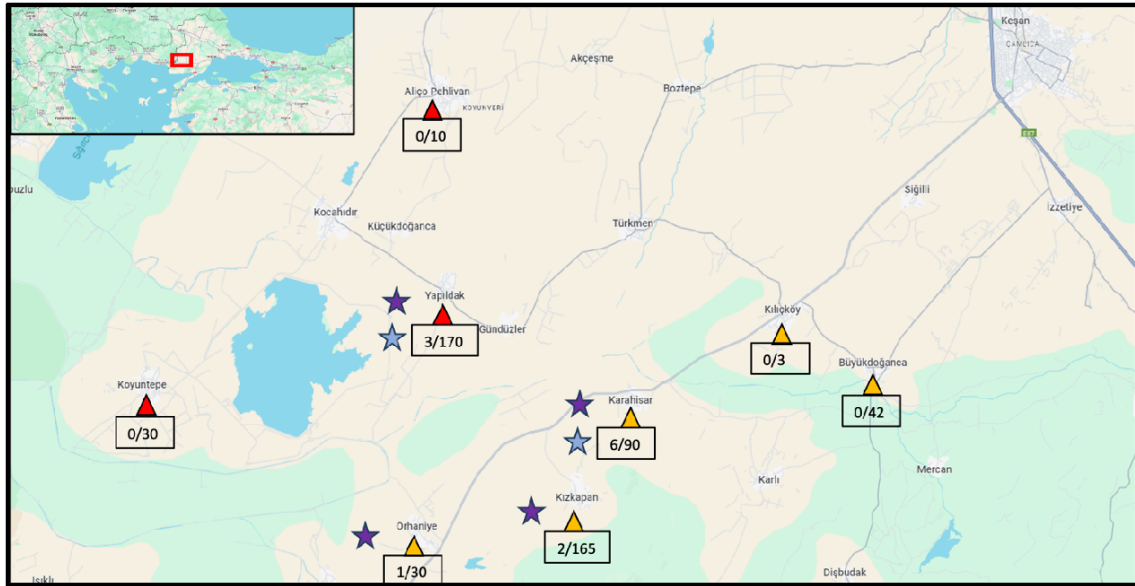


Figure 2. Positive pools detected in villages where ticks were collected (number of positive pools/number of collected ticks)

▲ Villages in the Keşan district of Edirne Province: Karahisar, Kılıçköy, Kızkapan, Orhaniye, Büyükdöğanca

▲ Villages in the İpsala district of Edirne Province: Yapıldak, Koyuntepe, Aliçopehlivan

★ Villages with *ompA* gene-positive pools

★ Villages with *gltA* gene-positive pools

Figure 2

See image above for figure legend