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Short communication

Borrelia miyamotoi in wild rodents from four different regions of Turkey

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ABSTRACT

Borrelia miyamotoi is a tick-borne zoonotic agent that causes hard tick-borne relapsing fever, an emerging disease in humans. Some small mammalian and bird species are reported to be reservoirs of *B. miyamotoi*. This study aims to examine *Borrelia* species present in rodents captured from rural areas of Turkey. Blood samples of rodents were initially screened with *Borrelia* 16S rRNA qPCR. The *Borrelia flaB* gene was subsequently amplified by conventional PCR, after which all positive samples were sequenced. *Borrelia miyamotoi* was observed in nine out of 536 blood samples (1.7%) collected from wild rodents. Phylogenetic analysis showed that all positive samples belonged to the European genotype clade of *B. miyamotoi*. PCR positivity was 5.3%, 3.7%, and 1.8% in *Apodemus uralensis, Apodemus flavicollis*, and *Myodes glareolus*, respectively. *Borrelia burgdorferi* sensu lato that causes Lyme borreliosis in humans could not be detected in the rodents. In this study, presence of *B. miyamotoi* DNA is reported for the first time in rodents in Turkey.

1. Introduction

Borrelia miyamotoi is a zoonotic microorganism in the *Borrelia* genus, and causes hard tick-borne relapsing fever (HTBRF) in humans. This bacterium was described for the first time in 1995 from *Ixodes persulcatus* ticks collected in Japan (Fukunaga et al., 1995). The vectors of tick-borne relapsing fever (TBRF) agents are generally argasid (soft) ticks of the genus *Ornithodoros*. Interestingly, it has been reported that some hard tick species of the genus *Ixodes*, generally vectors of *Borrelia burgdorferi* sensu lato (s.l.), are also the vectors of *B. miyamotoi* in Europe, Asia, and North America (Cutler et al., 2019). Some small mammalian species such as *Apodemus flavicollis, Apodemus sylvaticus, Myodes glareolus* and birds such as *Parus major, Carduelis chloris* have been suggested to be reservoirs of *B. miyamotoi* (Wagemarkers et al., 2017; Cerar et al., 2015; Szekeres et al., 2019; Talagrand-Reboul et al., 2018).

The *Borrelia* species that cause diseases in humans, belong to the Lyme Group and the Relapsing Fever Group. While some genospecies of *B. burgdorferi* s.l. cause Lyme borreliosis, the Relapsing Fever Group leads to several disorders in humans (Talagrand-Reboul et al., 2018; Cutler et al., 2019). Platonov et al. (2011) described *B. miyamotoi* for the first time in humans with HTBRF symptoms in Russia. Subsequently, many HTBRF cases that were caused by *B. miyamotoi* were reported. HTBRF is observed with a high fever and flu-like symptoms such as headache, myalgia, arthralgia, and malaise in humans. In some cases, erythema migrans and meningoencephalitis have also been reported (Cutler et al., 2019; Talagrand-Reboul et al., 2018).

Knowledge of the Lyme Group and Relapsing Fever Group *Borrelia* species vectors and reservoirs in Turkey is limited. This study aims to determine the presence and genetic diversity of *Borrelia* species in rodents from rural areas of Turkey.

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2. Materials and methods

2.1. Study setting

Rodent blood samples were available from a previous study conducted between 2010 and 2012 in the Burdur, Yozgat, Bartin, and Giresun provinces of Turkey (Fig. 1) (Oktem et al., 2014). Burdur is located in the Mediterranean biome and has a continental climate. Yozgat has a continental climate and vegetation typical of the Irano-Turanian subregion. Bartin and Giresun are located in the boreal (European-Siberian) belt.

2.2. Animal collection

A total of 536 rodents were captured from agricultural and forested areas in the four provinces using Sherman live traps and identified according to the standard taxonomic keys (Nagorsen and Peterson, 1980). The distribution of the captured rodent species by province is presented in Table 1. Blood samples were collected by cardiac puncture, frozen in liquid nitrogen, and transferred to the laboratory, where they were stored at −80 °C until further analysis. Permission to conduct this animal study was obtained from the Animal Ethics Committee of the Refik Saydam Hygiene Centre under reference no. 06.04.2009/11–27.

2.3. DNA extraction and PCR

DNA extraction from the blood samples was performed using the QIAamp DNA Blood Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were screened for *Borrelia* species with a real-time polymerase chain reaction (qPCR) targeting the *Borrelia* 16S rRNA gene using the p16Swt primers (fwd 5′-GGA TATAGTTAGAGATAATTATTCCCCGTTTG-3′, rev 5′-CATTACATGCTGGT AAC AGATAACAAGG-3′), probe (5′FAM-ACAGGTGCTGCATGGT–3′BHQ 1) and protocol previously reported by O'Rourke et al. (2013). Positive samples were further analysed with conventional PCR targeting a ~600 bp region of the *Borrelia* flagellin gene using Fla120F-Fla800R primers described by Dahmana et al. (2020). Reaction mixtures were prepared in a 50 µl volume, including 25 µl Amplitaq gold master mix (Applied Biosystems, Foster City, CA, USA), 1 µl (10 picomoles) of each primer, 5 µl of DNA template, and 18 µl of distilled water. *B.burgdorferi* B31 strain's DNA was used as the positive control and distilled water was used as the negative control for PCR reaction. All amplicons were visualised by electrophoresis in a 1.5% agarose gel. Positive amplification products were used for sequence analysis.

2.4. Sequence and phylogenetic analysis

PCR products were purified using NucleoFast 96 PCR ultrafiltration plates (Macherey Nagel EURL, Hoerdt, France) according to the manufacturer's instructions. The amplicons were sequenced using a Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City, CA, USA) with an ABI automated sequencer (Applied Biosystems, Foster City, CA, USA). The obtained electropherograms were assembled and edited using ChromasPro software (ChromasPro 1.7, Technelysium Pty Ltd., Tewantin, Australia). DNA sequences were compared with the records available in GenBank using the Basic Local Alignment Search Tool (Blast version 2.0). The data of obtained sequences were submitted to GenBank (OK044081–84). A *flaB* gene-based phylogenetic tree was inferred with MEGA7 using the maximum likelihood and Kimura 2-parameter method with 1000 bootstrap replicates (Kumar et al., 2016).

2.5. Statistical analysis

Statistical analysis was performed using Epi Info v.7 software. The frequency, infection prevalence and 95% confidence interval (CI) were evaluated using a Chi-square independence test. A *P*-value of < 0.05 was considered to be statistically significant.

3. Results

In this study, blood samples from 536 rodents trapped in four provinces of Turkey (Fig. 1) were analysed. According to morphological identification, the rodent species captured were as follows: *Apodemus flavicollis* ($n = 135$), *A. witherbyi* ($n = 93$), *A. uralensis* ($n = 56$), *A. mystacinus* ($n = 74$), *A. sylvaticus* ($n = 24$), *Myodes glareolus* ($n = 54$), *Chionomys roberti* ($n = 13$), *Microtus subterraneus* ($n = 4$), *Mus macedonicus* ($n = 57$) and *Microtus guentheri* ($n = 26$).

According to the qPCR screening results targeting the *Borrelia* 16S rRNA region, 11 out of 536 blood samples were determined to be positive. Amplification by conventional PCR targeting the *Borrelia* species *flaB* gene confirmed positivity in nine out of 11 qPCR positive samples.

When compared with *Borrelia* species sequences deposited in GenBank (accession numbers KT452930, KT932823, JF951386), it was observed that nine sequence results of the *FlaB* gene were between 99.7% and 100% identical with *B. miyamotoi* sequences. The features of the sequence data which were obtained from GenBank and compared with this study are presented in detail in the phylogenetic tree. Phylogenetic analysis with a high bootstrap value showed that all positive samples belonged to *B. miyamotoi* European genotype cluster (Fig. 2).

Borrelia miyamotoi was detected in 4.1% (95% CI: 1.5% - 8.7%) and 1.5% (95% CI: 0.3% −4.4%) of rodents captured in the Bartin and

Fig. 1. Provinces of Turkey where rodents were collected.

Table 1
Distribution of captured rodents according to province and *B. miyamotoi* prevalence.

Rodent species	*Borrelia miyamotoi* prevalence province				
	Bartin ($n = 147$) positive/total (%) (95% CI)	Giresun ($n = 196$) positive/total (%) (95% CI)	Yozgat ($n = 91$) positive/total (%) (95% CI)	Burdur ($n = 102$) positive/total (%) (95% CI)	Total ($n = 536$) positive/total (%) (95% CI)
Apodemus flavicollis ($n = 135$)	4/43 (9.3) (2.6–22.1)	1/66 (1.5) (0.04–8.2)	0/22 (0.0)	0/4 (0.0)	5/135 (3.7) (1.2–8.4)
Apodemus witherbyi ($n = 93$)	0/38 (0.0)	0/15 (0.0)	0/20 (0.0)	0/20 (0.0)	0/93 (0.0)
Apodemus uralensis ($n = 56$)	1/17 (5.8) (0.2–28.7)	2/39 (5.1) (0.6–17.3)	–	–	3/56 (5.4) (1.1–14.9)
Apodemus mystacinus ($n = 74$)	0/8 (0.0)	0/22 (0.0)	0/30 (0.0)	0/14 (0.0)	0/74 (0.0)
Apodemus sylvaticus ($n = 24$)	–	0/24 (0.0)	–	–	0/24 (0.0)
Myodes glareolus ($n = 54$)	1/41 (2.4) (0.1–12.9)	0/13 (0.0)	–	–	1/54 (1.9) (0.1–9.9)
Chionomys robertii ($n = 13$)	–	0/13 (0.0)	–	–	0/13 (0.0)
Microtus subterraneus ($n = 4$)	–	0/4 (0.0)	–	–	0/4 (0.0)
Mus macedonicus ($n = 57$)	–	–	–	0/57 (0.0)	0/57 (0.0)
Microtus guenterii ($n = 26$)	–	–	0/19 (0.0)	0/7 (0.0)	0/26 (0.0)
Total ($n = 536$)	**6/147 (4.1) (1.5–8.7)**	**3/196 (1.5) (0.3–4.4)**	**0/91 (0.0)**	**0/102 (0.0)**	**9/536 (1.7) (0.9–3.2)**

Giresun provinces, respectively (Table 1). None of the blood samples collected from the rodents trapped in Burdur and Yozgat were positive for *B. miyamotoi*. The total prevalence of *B. miyamotoi* in rodents was 1.7% (95% CI: 0.9%–3.2%). The infection prevalence was 5.3% in *A. uralensis* (95% CI: 1.2%–14.9%), 3.7% in *A. flavicollis* (95% CI: 1.1%–8.4%), and 1.8% in *M. glareolus* (95% CI: 0.1%–9.9%). Regionally, the highest *B. miyamotoi* prevalence was observed in *A. flavicollis* (9.3%) (95% CI: 2.6%–22.1%) in the Bartin province ($P = 0.18$). *Borrelia burgdorferi* s.l. was not detected in any rodents in the study.

4. Discussion

Rodents are reservoirs for many viral, parasitic, and bacterial zoonotic agents. The geographical distribution of these zoonotic agents varies according to rodent species and vectors. Rodent and vector movements are increasing in a globalising world dealing with climate change (Gratz, 2006). In recent years, rodent-borne agents such as the Hantaan virus, *Francisella tularensis, Bartonella* species, *Babesia microti, Hepatozoon* sp., *Sarcocystis* sp., and *Capillaria hepatica* have been reported in rodents in Turkey (Oktem et al., 2014; Çelebi et al., 2020, 2015; Usluca et al., 2019; Çelebi et al., 2014). No study on the presence of *Borrelia* species in rodents has been carried out in Turkey. However, studies on *Borrelia* species in Turkey have been conducted on the vector ticks and *B. burgdorferi* sensu stricto (s.s.), *B. garinii, B. afzelii, B. lusitaniae, B. valaisiana, B. miyamotoi,* and *B. spielmanii* have been detected in *I. ricinus* ticks, and *B. turcica* has been detected in *Hyalomma aegyptium* ticks (Güner et al., 2003, 2004; Gargili et al., 2010; Karasartova et al., 2018; Sakakibara et al., 2016). In this study, *B. miyamotoi* was identified for the first time in wild rodents from rural areas in Turkey.

Borrelia miyamotoi positivity has been reported at rates varying between 0.05% and 9% in studies conducted on rodents in many European countries (Ruyts et al., 2017; Hamsikova et al., 2017; Kalmar et al., 2019; Wagemakers et al., 2017; Cerar et al., 2015; Szekeres et al., 2019; Casson et al., 2014; Tadin et al., 2016). In this study, 1.7% of rodents were positive for *B. miyamotoi*. In Europe, *B. miyamotoi* has been reported in *A. flavicollis, A. sylvaticus, Apodemus agrarius, C. glareolus, Microtus arvalis,* and *Sciurus vulgaris* (Tadin et al., 2016; Ruyts et al., 2017; Hamsikova et al., 2017; Wagemakers et al., 2017), whereas in the present study the bacterium was detected, except for *A. flavicollis* and *M. glareolus*, in *A. uralensis* for the first time.

Borrelia miyamotoi is divided into three genotypes, European, Asian, and American, and their vector ticks are *I. ricinus, I. persulcatus,* and *I. scapularis* or *I. pacificus,* respectively. It is presumed that the genetic features of this agent depend on the vector species, so *B. miyamotoi* genotypes differ regionally (Mukhacheva et al., 2015; Geller et al., 2012; Diuk-Wasser et al., 2016). This study was conducted in the Asian part of Turkey and the *B. miyamotoi* European genotype was detected in rodents in the rural areas of the Bartin and Giresun provinces, which are located in the boreal (European-Siberian) belt. The Bartin and Giresun provinces are the regions where *I. ricinus*, the vector of the *B. miyamotoi* European genotype, is most frequently reported in Turkey. The provinces of Yozgat and Burdur, where *B. miyamotoi* could not be determined in rodents, are the regions where *I. ricinus* are rarely observed (Estrada-Peña et al., 2017).

The 16S rRNA, Flagellin (*flaB*) gene, the outer membrane protein *p66*, and the *glpQ* gene regions have been used in the molecular characterisation of *B. miyamotoi* (Mukhacheva et al., 2015). The phylogenetic tree of the Flagellin (*flaB*) gene revealed that all *B. miyamotoi* identified in the present study share high similarities with other European genotypes that have been reported in humans, ticks, and rodents (Fig. 2).

Single infections with *B. burgdorferi* s.l. and Relapsing Fever Group *Borrelia* species or both have been reported in rodents (Hamsikova et al., 2017; Cerar et al., 2015; Kalmar et al., 2019). *Borrelia burgdorferi* s.l. were not detected in this study. Liang et al. (2020) reported that *B. burgdorferi* s.s. is rapidly eliminated from rodent blood and localised to tissues, whereas the infection of *Borrelia persica* from the TBRF group persists in rodent blood for a long time. Sinsky and Piesman (1989) confirmed that ear punch biopsy samples are an effective clinical material in determining the *B. burgdorferi* s.l. group in rodents. As rodent blood was used as a clinical sample, the density of *B. burgdorferi* s.l. in the blood might be below the detection limit of the PCR method used in this study. The limitation of our study is that while rodent blood is a clinical sample that can be used to identify the HTBRF group *Borrelia* spp., rodent skin or ear biopsy samples, which are important clinical samples for the detection of *B. burgdorferi* s.l., were not used.

TBRF is an emerging disease, and no cases have been reported in Turkey. However, *B. miyamotoi*, which causes relapsing fever in humans, has been reported in rodents (this study) and in an *I. ricinus* tick (Sakakibara et al., 2016). More comprehensive studies should be carried out to determine the prevalence of *B. miyamotoi* in *I. ricinus* ticks that are observed in some parts of Turkey.

CRediT authorship contribution statement

Bekir Çelebi: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **Derya Karataş Yeni:** Formal analysis, Data curation, Software. **Yusuf Yılmaz:** Investigation, Visualization. **Ferhat Matur:** Investigation, Data curation. **Cahit Babür:** Investigation, Data curation. **Mehmet Ali Öktem:** Investigation, Data

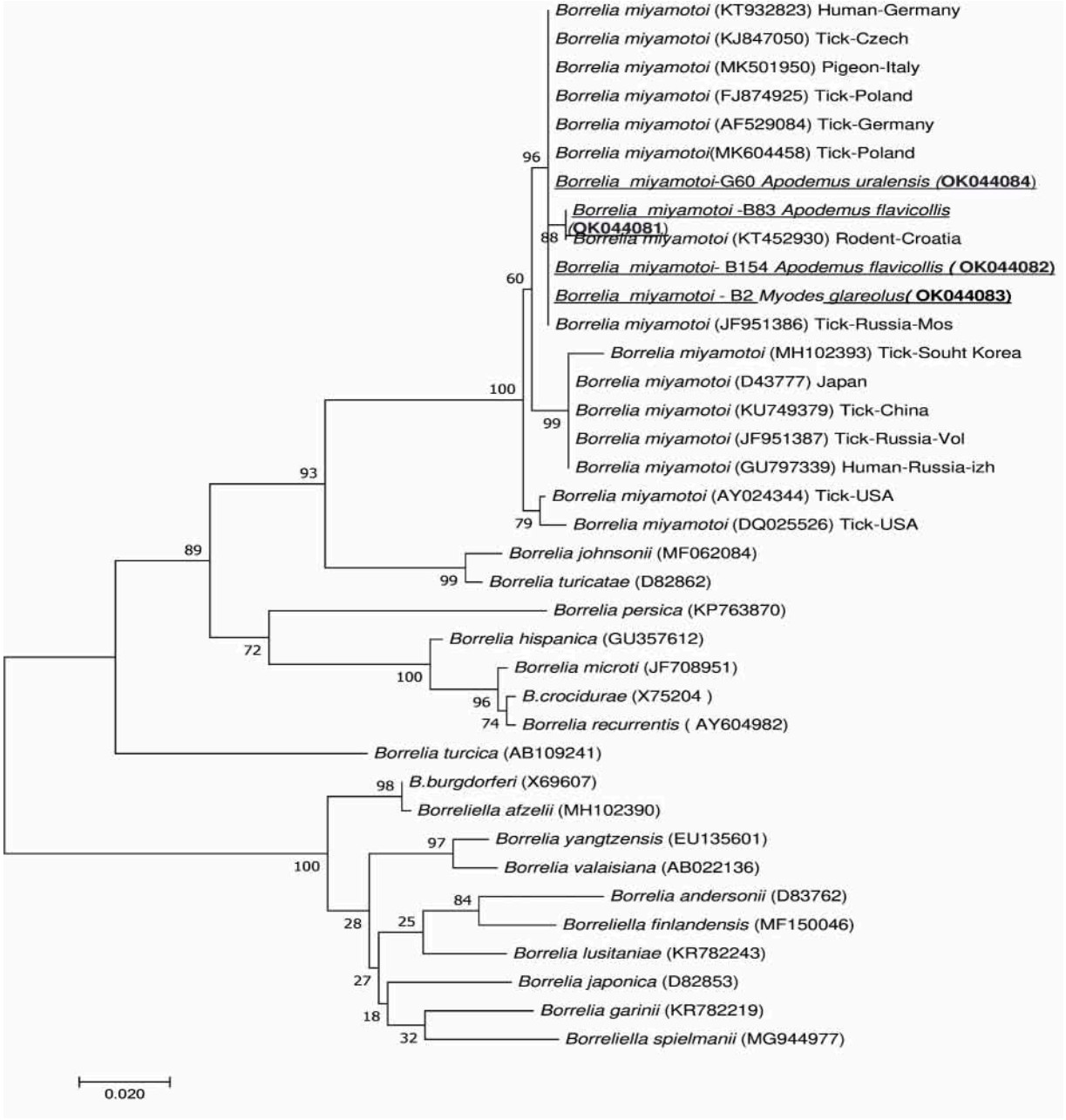

Fig. 2. The *FlaB*-gene based phylogenetic tree highlighting the position of *Borrelia miyamotoi* (bold) detected in this study, relative to 33 other *Borrelia* spp. nucleotide sequences. Sequences were aligned using ClustalW. The evolutionary history was inferred using the Maximum Likelihood Method and the Kimura 2-parameter within the MEGA software version 7.0. Codon positions included were first+second+third+noncoding. There were a total of 520 positions in the final dataset. Values at the nodes are bootstrap replicates obtained by repeating the analysis 1000 times. The scale bar represents 0.02% sequence divergence. GenBank accession numbers are presented in parentheses.

curation. **Mustafa Sözen:** Investigation, Data curation. **Ahmet Karataş:** Investigation, Data curation. **Didier Raoult:** Funding acquisition, Resources. **Oleg Mediannikov:** Methodology, Supervision, Validation. **Pierre Edouard Fournier:** Project administration, Supervision, Writing – review & editing.

Data availability

The authors do not have permission to share data.

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