Questing *Ixodes ricinus* ticks (Acari, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in an urban area of north-eastern Poland

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Abstract

Green areas located within large cities, as natural ecotypes, are a convenient habitat for ticks and their use as recreational areas is associated with the potential risk of acquiring tick-borne diseases. This study estimated the *I. ricinus* tick density, prevalence of infection with *Borrelia* species and the diversity of these bacteria in a green urban area (Olsztyn) of north-eastern Poland, an endemic region of tick-borne diseases. The ticks were collected during spring and autumn of 2015, at sites differing in the degree of human pressure and habitat. *Borrelia* species detection, typing and a molecular phylogenetic analysis were carried out based on the sequenced *flaB* gene. The overall mean abundance of *I. ricinus* was 2.0 ± 1.55 ticks per 100 m². The density of *I. ricinus* did not vary significantly between sites. According to semi-qualitative tick abundance categories, the collection sites were classified as ‘very low’ and ‘low’ tick abundance category. The overall infection rate of *I. ricinus* with *Borrelia* spirochaetes was 27.4%. The infection rate of adult ticks (42.0%) was three times higher than with nymphs (14.3%). Based on the restriction patterns and sequencing, *B. afzelii* (93.1%; 27/29), *B. valaisiana* 3.5% (1/29) and *B. miyamotoi* (3.5%; 1/29), related to the relapsing fever (RF) spirochaetes, were detected. No co-infections were found. *Borrelia miyamotoi*, detected for the first time in ticks in the north-eastern urban areas of Poland, was identical to isolates described as European-type. The *Borrelia* spirochaete infection rate of *I. ricinus* ticks in an urban area indicated a high risk of LB. Physicians should also be aware of *B. miyamotoi* infections among patients with a history of tick-bites in north-eastern Poland.

Keywords *Ixodes ricinus* · *Borrelia burgdorferi* sensu lato complex · *Borrelia miyamotoi* · Urban area · Poland

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Introduction

In Europe, the *Ixodes ricinus* tick is the most widespread reservoir and vector of many pathogenic agents (viruses, bacteria and protozoa) of medical and veterinary importance (Medlock et al. 2013; Parola and Raoult 2001; Parola and Paddock 2018). Due to the increasing tick-borne disease (TBDs) incidence rate and the confirmed pathogenicity of new microorganism species transmitted by ticks, most of the tick-transmitted diseases are classified as emerging infectious diseases (EIDs) (Jongejan and Uilenberg 2004; Vorou et al. 2007; Vu Hai et al. 2014). The most commonly diagnosed tick-borne disease in humans is Lyme borreliosis (LB), caused by spirochaetes of the *Borrelia burgdorferi* complex (Medlock et al., 2018). In this group comprising 20 species, five (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. bavariensis* and *B. spielmanii*) are regarded as human pathogens (Jungnick et al. 2015; Margos et al. 2011). The pathogenicity of *B. valaisana*, *B. bissetti* and *B. lusitaniae* is also not excluded (Derdáková and Lenčáková 2005; Diza et al. 2004; Jungnick et al. 2015; Margos et al. 2017; Stanek and Reiter 2011). Different *Borrelia* species and/or genotypes implicated in the infection cause a broad spectrum of the clinical picture of LB, covering various tissues and organs, including skin, joints, heart and nervous system, with the course of illness from acute to chronic (Stanek and Reiter 2011). In Europe, 85,999 LB cases are recorded per year (Pritt et al. 2016). In Poland, in 2015–2017, LB was diagnosed in approximately 19,000 patients a year. The largest number of LB cases were found in north-eastern Poland (the Warmia-Mazury province), where the incident rate was almost twice as high as in the rest of the country (NIH-PZH). The involvement of *I. ricinus* ticks in *B. miyamotoi* transmission—a species closely related to LB *Borrelia* spirochaetes and belonging to the relapsing fever (RF) group of *Borrelia* has also been confirmed (Fukunaga et al. 1995; Richter et al. 2003). Until 2011, when the first symptomatic infection in human was recorded in Russia, *B. miyamotoi* had been regarded as a non-pathogenic species (Platonov et al. 2011). Since that time, subsequent cases of infection with *B. miyamotoi* in humans have been noted in the USA (Krause et al. 2013; Molloy et al. 2015), Europe (The Netherlands, Germany) (Hansford et al. 2015; Hovius et al. 2013; Jahfari et al. 2016) and Japan (Sato et al. 2014). Symptoms of infection caused by *B. miyamotoi* (*Borrelia miyamotoi* disease—BMD) are non-specific and diverse. In patients, BMD manifests itself with high fever (up to 40 °C), fatigue, headaches, chills, myalgia, arthralgia and nausea; meningoencephalitis may also develop (Platonov et al. 2011).

In many European cities, the tendency to preserve green areas, such as parks and forests, undeveloped squares, old cemeteries and gardens, creates favourable conditions for the development of populations of small and medium-size mammals. This group of animals, as hosts, help to maintain and spread tick populations in urban areas. On the other hand, green city areas are also used by urban dwellers and their companion animals for recreational activities, which increases the risk of acquiring a tick-borne disease through regular exposure to tick bites (Uspensky 2014). One of the measures for estimating the risk of tick-borne diseases is monitoring questing tick distribution and their population density in an area, and an assessment of the level of their infection with pathogens (Kilpatrick et al. 2017; Vu Hai et al. 2014). This approach is of key importance for undertaking the appropriate prophylactic and educational measures.

Due to the high LB and tick-borne encephalitis incidence rate in the inhabitants, the region of north-eastern Poland is regarded as endemic for tick-borne diseases (NIH-PZH). The risk of acquiring tick-borne diseases in this area of Poland is usually connected with staying in an area of low anthropopressure—forest ecotypes or rural areas—the habitats

typical of *I. ricinus* ticks and their hosts (Pawelczyk and Sinski 2001, 2004; Stańczak et al. 2000; Stańczak and Kubica-Biernat 1999). Due to the lack of data about the ticks and tick-borne pathogens in urban areas in this region, the aim of the study was to determine the role of *I. ricinus* ticks as a vector of microorganisms of the *Borrelia* genus in city areas of north-eastern Poland.

Materials and methods

Study area, tick collection

The study was conducted in three green recreational areas in the city of Olsztyn (53°46′23″N 20°28′34″E; 88.0 km²; 173 444 residents), the capital of the province of Warmia-Mazury (north-eastern Poland) (Fig. 1). The tick collection sites (surfaces of 350–400 m²) differed in the degree of human pressure and habitat (Table 1). At each site, the questing *I. ricinus* ticks were collected between April–May and September 2015 during the daytime between 9 a.m. and 4 p.m. by one person for at least 30 min using the standard flagging method. In the laboratory, the collected ticks were identified by species, sex and developmental stage using taxonomic keys (Nowak-Chmura 2013; Siuda 1993) and preserved in 70% ethanol for further molecular investigation. For each collection site, tick abundance was calculated as the number of specimens collected per 100 m² for each individual flagging event.

DNA extraction

Prior to DNA extraction, the ticks were air-dried for several minutes and then individually cut with a sterile scalpel. Genomic DNA extraction from ticks was carried out by universal kit Sherlock AX (A&A Biotechnology, Poland) according to the manufacturer's instructions. DNA was eluted in 40 µL of TE buffer and stored at –20 °C for further analysis.

Fig. 1 Locations of the study area in north-eastern Poland

Table 1 Characteristics of collection sites and mean density of *Ixodes ricinus* in the city of Olsztyn, north-eastern Poland

Locality	Geographical coordinates	Anthropopressure degree	Groups exposed to contact with ticks	Habitat	Tick density (mean number of specimens per 100 m ² ± SD)*
Olsztyn-Brzeziny	53°44'46.9"N 20°27'34.8"E	Low the area along a street with a pedestrian-bicycle sidewalk, near the hotel	Pedestrians, tourists	Abandoned grassy field separated from the street by a zone of tall trees and shrubs	1.0 ± 1.09
Olsztyn-Mazurskie	53°45'39.4"N 20°31'06.1"E	Medium an area along the pedestrian-bicycle path is used for recreation (walking with dogs, active sports)	Residents of a nearby housing estate, pedestrians	Meadows sparsely covered by trees or bushes, near Lake Skanda	2.5 ± 1.36
Olsztyn-Kortowo	53°45'13"N 20°27'20"E	High part of the city park located on the university campus, area along the footpath and bicycle path, the nearby beach, used as a communication route between university buildings and as a recreational area (picnic, walking with dogs, sports activities)	Students, residents of a nearby housing estate, pedestrians; during summer holidays: tourists, sunbathers and children—participants of summer camps and sports camps	Recreational city park with grassy areas and covered by trees or bushes, near Lake Kortowskie	2.1 ± 2.05

* $\chi^2 = 2.528$, df = 2, p = 0.28

Detection and identification of spirochaete DNA by the nested PCR–RFLP method

The presence of *Borrelia* spirochaetes in tick genomic DNA samples was confirmed by the nested polymerase chain reaction (PCR) method using two sets of primers specific to the flagellin gene (*flaB*): outer—132f/905r (774 bp) and inner—220f/823r (604 bp) (Wodecka et al. 2010). The 25 µL of PCR mixture contained 12.5 µL of 2×PCR Master Mix Plus (0.1 U/µL of *Taq* polymerase supplied in a PCR buffer, 4 mM of MgCl₂ and 0.5 mM of each dNTPs); (A&A Biotechnology, Poland), 0.5 µL of each primer (10 mM), 10.5 µL sterile nuclease-free water and 1 µL of template DNA or 1 µL of the outer PCR product for nested PCR. The thermal conditions of PCRs were: 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 53 °C (54 °C for inner primers), 30 s at 72 °C and a final extension of 7 min at 72 °C (iCycler MyiQ, Bio-Rad, USA). DNA isolated from *B. afzelii* positive *I. ricinus* tick (confirmed by sequencing) and nuclease-free water were run in each analysis as positive and negative controls. Nested PCR products were separated on 2% agarose gels stained with ethidium bromide and visualized using GelDocXR (Bio-Rad, USA).

To the identification of *Borrelia* species, the restriction fragment length polymorphism (RFLP) method was used. The positive inner PCR products (604 bp) were digested with the restriction enzyme HpyF3I (DdeI) (ThermoFisher Scientific, USA) according to the manufacturer's instructions. Restriction fragments were separated on 3% agarose gel stained with ethidium bromide. Recognition of the species was based on the RFLP patterns given by Wodecka 2011 and Wodecka et al. 2010.

DNA sequencing

To confirm PCR–RFLP results chosen, positive inner PCR products were purified using the Clean Up purification kit (A&A Biotechnology, Poland) according to the manufacturer's protocol and sequenced bi-directionally with inner primers (220f/823r) (Macrogen Europe, the Netherlands). The obtained nucleotide sequences were edited in BioEdit software (Hall 1999) and compared with data registered in the GenBank database (<http://www.ncbi.nih.gov/Genbank/index.html>) using the BLAST-NCBI program (<http://www.ncbi.nlm.nih.gov/BLAST/>). Consensus *Borrelia flaB* gene sequences were deposited in the GenBank database and registered under the accession numbers MK059480–MK059484.

Phylogenetic analysis

Nine representative *Borrelia* sequences obtained from the collected *I. ricinus* ticks and the most similar chosen reference sequences from GenBank were used in phylogenetic analysis. The phylogram was constructed using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The topology of the phylogenetic tree was evaluated using the bootstrap method with 1000 replicates. Phylogenetic analysis was conducted using MEGA v.6.06 software (Tamura et al. 2013).

Statistical analysis

The differences in the abundance of *I. ricinus* ticks between collection sites were analysed using the one way non-parametric ANOVA (Kruskal–Wallis H test). A *Chi square* test was

used to compare the infection prevalence of *Borrelia* species between developmental stages of ticks and study areas. In both tests, the differences were considered to be statistically significant when the p value was less than 0.05. The analysis was conducted using the software package SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL).

Results

Tick abundance

Among 106 collected questing *I. ricinus* ticks, 50 (48%) adult specimens (30 females and 20 males) and 56 (52%) nymphs were identified. The overall mean abundance was 2.0 ± 1.55 ticks per 100 m². The density of *I. ricinus* did not vary significantly ($\chi^2 = 2.528$; $df = 2$; $p = 0.28$) between sites and it ranged from 1.0 ± 1.09 (Brzeziny) to 2.5 ± 1.36 (Mazurskie) ticks per 100 m² (Table 1). According to semi-qualitative tick abundance categories (Schwarz et al. 2009), the collection sites were classified as ‘very low tick abundance’ (Brzeziny and Kortowo) or ‘low tick abundance’ (Mazurskie).

Prevalence of spirochaetes

The overall prevalence of *Borrelia* spp. and *B. miyamotoi* spirochaetes in *I. ricinus* was 27.4% (29/106) (Table 2). The infection rate of adult ticks (42.0%; 21/50) was three times higher than nymphs (14.3%; 8/56). Females showed an infection rate of 43.3% (13/30) whereas 40% (8/20) of males were *Borrelia*-positive. The differences in infection rates between tick developmental stages were statistically significant ($\chi^2 = 10.276$; $df = 2$; $p = 0.006$). *Borrelia* species were detected in *I. ricinus* from all study sites. The infection rate did not differ between collection sites ($\chi^2 = 2.055$; $df = 2$; $p = 0.36$) and ranged from 18.2% (Kortowo) to 33.3% (Brzeziny) (Table 2).

Table 2 Infection rates of *Ixodes ricinus* ticks with *Borrelia* species according to the tick developmental stages and locality in urban areas of Olsztyn, north-eastern Poland

	No. (%) of collected	No. (%) positive for each <i>Borrelia</i> species			Total no. (%) of infected
		<i>B. afzelii</i>	<i>B. valaisiana</i>	<i>B. miyamotoi</i>	
Stage					
Females	30 (28.3)	11 (84.6)	1 (7.7)	1 (7.7)	13 (43.3)*
Males	20 (18.9)	8 (100)	0 (0)	0 (0)	8 (40.0)*
Nymphs	56 (52.8)	8 (100)	0 (0)	0 (0)	8 (14.3)*
Locality					
Brzeziny	12 (11.3)	3 (75.0)	0 (0)	1(25.0)	4 (33.3)**
Mazurskie	61 (57.5)	18 (94.7)	1 (5.3)	0 (0)	19 (31.1)**
Kortowo	33 (31.1)	6 (100)	0 (0)	0 (0)	6 (18.2)**
Total	106 (100)	27 (93.1)	1 (3.5)	1 (3.5)	29 (27.4)

*($\chi^2 = 10.276$, $df = 2$, $p = 0.006$); **($\chi^2 = 2.055$, $df = 2$, $p = 0.36$)

Genotyping and diversity of spirochaetes

Based on the restriction patterns obtained after digestion, 604 bp fragments of the *flaB* gene *B. afzelii* (305, 165, 94, 40 bp; pattern II), *B. valaisiana* (188, 135, 117, 92, 72 bp; pattern VI) and *B. miyamotoi* (512, 86 bp; pattern IX) were detected (Fig. 2). The dominant pathogen species was *B. afzelii* (93.1%, 27/29). *B. valaisiana* was identified in 3.5% (1/29) and *B. miyamotoi* in 3.5% (1/29) (Table 2). No co-infections were found.

To confirm PCR–RFLP results, positive inner PCR of *B. afzelii* (n=7), *B. valaisiana* (n=1) and *B. miyamotoi* (n=1) products were sequenced. Among *B. afzelii*, three variants of *flaB* gene sequences were identified. One of the most frequent variants (BaOL1, MK059480), recognized in four isolates, was identical with the pathogenic BO23 *B. afzelii* strain (CP018262) isolated from the skin of German patients and sequences detected in the blood of *Apodemus flavicolis* (KY626319) and questing *I. ricinus* from Poland (Fig. 3). In other variants (BaOL2, MK059482 and BaOL3, MK059481), nucleotide substitutions were noted. In the BaOL2 variant, substitution in one nucleotide position (1 SNP) did not change the amino-acid sequence. In BaOL3, the variant nucleotide variation in position three (3 SNP) changed the amino-acid flagellin sequence. *FlaB* sequences of *B. valaisiana* (MK059484) were identical to BvV2 variant (MF150078) of this species detected in *I. ricinus* from Białowieża National Park in north-eastern Poland and displayed high similarity to sequences from questing *I. ricinus* in Poland and Turkey (Fig. 3). The *B. miyamotoi flaB* gene sequence (MK059483) was identical to the sequences of isolate Mos-80(1) of *B. miyamotoi* from Russian *I. ricinus* designated as European-type (Platonov et al. 2011; Takano et al. 2014). On the phylogram, this sequence (clustered together with other sequences) was detected in *I. ricinus* from different parts of Poland and Czech Republic (Fig. 3).

Fig. 2 The RFLP patterns obtained after *DdeI* enzyme digestion of positive nested-PCR products (604 bp): M-DNA size marker; B.a. *Borrelia afzelii*; B.m. *B. miyamotoi*; B.v. *B. valaisiana*

Fig. 3 Phylogram constructed based on partial sequences of *flaB* gene by using the Maximum Likelihood method with the Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers at the nodes of the tree indicated bootstrap values (1000 replicates). The analysis involved 23 nucleotide sequences. There were a total of 527 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The sequences obtained in this study were labelled with black symbols. B.a. *Borrelia afzelii*, B.v. *B. valaisiana*, B.m. *B. miyamotoi*

Discussion

Areas situated within the administrative boundaries of large European cities, have repeatedly been indicated as an environment favourable to *I. ricinus* ticks (Didyk et al. 2017; Hansford et al. 2017; Kowalec et al. 2017; Uspensky 2014). The average tick population density in habitats located in urban areas is nearly 2–3 times lower compared to natural ecotypes (Dobson et al. 2011; Hamšíková et al. 2017; Kowalec et al. 2017; Kubiak and Dziekońska-Rynko 2006). The results of our study indicate that urban areas in north-eastern Poland are not safe regarding the risk of LB and other TBDs. Regardless of the degree of anthropopressure, the collection sites in Olsztyn were classified as “low” or “very low”

in tick abundance (Schwarz et al. 2009), with the mean tick *I. ricinus* population density ranging from 1.0 to 2.1 specimens per 100 m². Such a low population density must be associated with the specimen collection sites located in typical recreational areas and not connected with large forest complexes, which considerably limits the accessibility of medium-size and large *I. ricinus* hosts. Limiting access to hosts has also been identified as a factor which reduced the density of *I. ricinus* ticks in parks in Warsaw by half compared to a large forest complex situated within the administrative boundaries of the city (Kowalec et al. 2017). Apart from the tick abundance, the risk of contracting TBDs is affected by human behaviour patterns, which bring people in contact with the habitats resided by ticks (Kilpatrick et al. 2017; Randolph and EDEN-TBD sub-project team 2010; Rizzoli et al. 2014). Exposure to *I. ricinus* ticks and infection with TBDs is associated with fresh air activities (Lane et al., 2004; Padgett and Bonilla 2011; Randolph and EDEN-TBD sub-project team 2010; Wierzbicka et al. 2016). In the green areas covered by our study, these activities include walking, jogging, walking pets or behaviour which necessitates direct contact with the ground and forest litter, such as resting on the grass or using wooden logs or tree trunks as picnic sites. The risk of contracting TBDs is also significantly affected by the level of infection of ticks and the species composition of the pathogens (Kilpatrick et al. 2017). Infection of *I. ricinus* with spirochaetes of the *B. burgdorferii* s.l. complex is diverse, not only in Europe (3.6–19.3%) (Strnad et al. 2014), but also within a specific country. In Poland, the *I. ricinus* infection rate varies from 1.2 to 12.4% depending on the region (Karbowski et al. 2018). Data on the level of infection of the population of *I. ricinus* with *B. burgdorferi* spirochaetes in north-eastern Poland date from the turn of the 21st century. At that time, it was indicated that prevalence of *Borrelia* in *I. ricinus* ranges from 10.3 to 23.1%, depending on the study year (Pawelczyk and Sinski 2001, 2004; Stańczak et al. 2000; Stańczak and Kubica-Biernat 1999). Only *B. garinii* and *B. afzelii* were found in this area, which was probably due to the identification methods applied (Stańczak et al. 2000). The level of 27% of infected *I. ricinus* in urban areas of north-eastern Poland that we established in our study is much higher and it did not differ significantly between the study sites. The level of infection of *I. ricinus* ticks in Olsztyn was also much higher compared to the green areas in other large cities, such as Kiev 4% (Didyk et al. 2017), Warsaw 10.9% (Kowalec et al. 2017), Luxembourg 11.3% (Reye et al. 2010), Salisbury 18.1% (Hansford et al. 2017). A comparable or higher level was recorded only in large cities in Germany—Hanover (22.7%) (Tappe et al. 2014) and Hamburg (34.1%) (May et al. 2015). The data for Olsztyn confirmed the general trend that the level of infection of adult ticks is much higher than that of nymphs (Karbowski et al. 2018; Strnad et al. 2014). The infection rate of adult ticks (42.0%) was three times higher than nymphs (14.3%).

However, human impact on the environment in urban areas and the connected lower tick abundance do not have an effect on the prevalence of *Borrelia* spirochaetes *I. ricinus* (Kowalec et al. 2017). Comparison of populations of *I. ricinus* in urban areas in central Poland and natural areas in eastern Poland indicated a similar level of spirochaetes infections (10.9% vs. 12.2%, respectively) (Kowalec et al. 2017). The diversity of spirochaetes in *I. ricinus* ticks in these two types of environment was also comparable. However, our study did not demonstrate such a rich species composition, which is probably caused by the small number of ticks taken for examination. Among nine species from the *B. burgdorferii* s.l. complex recorded in Europe and six identified by Kowalec et al. (2017) in natural habitats in eastern Poland, the *I. ricinus* ticks in Olsztyn were found to be infected only with *B. afzelii* and *B. valaisiana*. We also confirmed a high percentage of a human pathogen *B. afzelii* (93.1%) in the infected ticks, which suggests a high risk of LB with skin symptoms of infection. A meta-analysis of 2010–2016 data concerning the species composition of

Borrelia spirochaetes in ticks from 23 European countries, indicated *B. afzelii* to be the most commonly identified species (Strnad et al. 2014). *B. afzelii* was the dominant species in the population of infected *I. ricinus* ticks in the green areas of Warsaw (Kowalec et al. 2017), Hanover (Tappe et al. 2014), Kiev (Didyk et al. 2017) and Luxembourg (Reye et al. 2010). However, the second most common species—*B. garinii*, which dominates among the infected ticks in forested areas in Bratislava (Chvostáč et al. 2018) and Salisbury (Hansford et al. 2017) and in natural ecotypes in the east of Poland (Kowalec et al. 2017), was not detected. These differences are probably associated with the group of *I. ricinus* available hosts. *B. afzelii* is associated with rodents, whereas *B. garinii* with birds. Birds are also involved in the transmission of *B. valaisiana*—a species whose human pathogenicity is poorly documented but detected in questing ticks from green areas of Olsztyn and other cities (Chvostáč et al. 2018; Hansford et al. 2017; Kowalec et al. 2017; Tappe et al. 2014).

Of particular interest is the detection of *B. miyamotoi*. We revealed the occurrence of this pathogen in Olsztyn for the first time and it was confirmed in the subsequent year of the study (unpublished data). The sequence of the *B. miyamotoi* *flaB* gene fragment identified in our study is identical to the sequences in European-type *B. miyamotoi* (Platonov et al. 2011; Takano et al. 2014). According to Kowalec et al. (2017), within European-type *B. miyamotoi* there may be a specific Polish strain of this pathogen, which is also present in *I. ricinus* in the area of Olsztyn. The low prevalence of *B. miyamotoi* (3.5%, 1/106) estimated in our study is typical of Europe. The *I. ricinus* infection rate in many European countries ranges from 0.16% in Portugal (Nunes et al. 2015) to 4.8% in Hungary (Szekeres et al. 2015). In Poland, *B. miyamotoi* was identified in *I. ricinus* in recreational areas in Szczecin (Wodecka et al. 2010) and Warsaw (Kowalec et al. 2017) and in natural habitats of Lower Silesia (Kiewra et al. 2014) and eastern Poland (Kowalec et al. 2017) with a low prevalence from 0.5 to 3.9%. The relatively low infection rate of *B. miyamotoi* in questing *Ixodes* spp. can be caused by an adverse effect of spirochaetes on the survival rate of infected ticks or by the low rate of ticks acquiring it when feeding on infected hosts (Barbour et al. 2009; Wagemakers et al. 2015). However, it does not rule out the possibility of the incidence of BMD in areas endemic to LB and other TBDs, especially since the co-infection of *B. miyamotoi* with other pathogens has been observed, both in vectors (Kiewra et al. 2014; Wagemakers et al. 2015) and in patients (Platonov et al. 2011). Fiecek and Chmielewski (2017) note that a BMD should be considered in patients with high fever or neurological symptoms, in whom anti-*Borrelia* antibodies are detected only in serum. This group of neurological patients do not meet the criteria for neuroborreliosis. In Poland, most cases of neuroborreliosis are registered as probable cases with unconfirmed diagnosis by the presence of anti-*Borrelia* antibodies in cerebrospinal fluid (CSF) (Fiecek and Chmielewski 2017).

Conclusion

The high *Borrelia* infection rate of *I. ricinus* ticks revealed in Olsztyn confirms that green areas in the cities located in areas endemic to TBDs, such as north-eastern Poland, is linked to a high risk of LB, despite a low *I. ricinus* abundance. Domination of the human pathogen *B. afzelii* among infected *I. ricinus* ticks suggests that residents with borreliosis will primarily reveal skin symptoms of this disease. Although there is a low prevalence of the pathogenic species of *B. miyamotoi*, cases of BMD should be considered in patients declaring tick bites, especially in patients with high fever and meningoencephalitis, without

anti-*Borrelia* antibodies in CSF. A comprehensive study must be conducted in north-eastern Poland to determine the *B. miyamotoi* prevalence in *I. ricinus* ticks and take measures to educate and raise awareness of TBD presence other than LB, not only among residents, but also among physicians.

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