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Molecular screening for *Bartonella henselae* and *Borrelia burgdorferi* sensu lato co-existence within *Ixodes ricinus* populations in central and eastern parts of Poland

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Abstract

The presented study aimed at establishing the prevalence and co-infection rates of *Bartonella henselae* and *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected from the central and eastern parts of Poland. The common tick individuals were gathered in the years 2008-2009. Questing ticks were sampled by dragging a white woollen flag over lower vegetation at 17 localities within diverse types of habitats: urban recreational green areas (city parks and squares), suburban forests and rural woodlands throughout the investigated regions of Poland. Detection of *B. henselae* in tested tick specimens was based on PCR amplification of the citrate synthase (*gltA*) gene, while screening for the presence of *B. burgdorferi* s.l. DNA was carried out by analyzing fragments of two genes: the flagellin (*fla*) and outer surface protein A (*ospA*). A total number of 1,571 *I. ricinus* ticks were sampled: 865 (55.1%) nymphs, 377 females (24.0%) and 329 males (20.9%). The application of PCR assays revealed that 76 (4.8%) tick samples were *B. henselae*-positive, *B. burgdorferi* s.l. DNA was detected in 194 specimens (12.3%), whereas the co-existence of these pathogens was evidenced in 22 tested ticks (1.4%). Furthermore, the occurrence of bartonellae and co-circulation of analysed microorganisms in *I. ricinus* was affirmed only within adult individuals, while presence of the screened spirochetes was ascertained in both nymphal and adult ticks. It should be stressed that the suburban woods of Warsaw and rural forests in Warsaw County characterized the highest prevalence levels of dual infection with investigated tick-borne pathogens, whereas the lowest co-infection rates were recorded in tick populations inhabiting rural forests in Płock County and forested areas in Korczew-Mogielnica (within the Nadbużański Landscape Park).

Key words

Bartonella henselae, *Borrelia burgdorferi* sensu lato, *Ixodes ricinus*, co-infection, molecular diagnostics

INTRODUCTION

Bartonella henselae is a polymorphic, fastidious and intracellular Gram-negative bacterium causing a broad range of diverse human infections, including the cat scratch disease (SCD), and less frequently, bacillary angiomatosis, peliosis hepatitis, bacteraemia, endocarditis and neuroretinitis, emerging especially in immunocompromised patients [1, 2, 3]. This worldwide distributed zoonotic pathogen is incidentally transmitted from its major reservoir host (the cat) to humans [4, 5, 6]. Symptoms of *B. henselae* infection in immunocompetent people are usually limited to a unilateral localized lymphadenopathy and fever occurring during 2-3 weeks after a feline scratch or bite. Conversely, clinico-pathological forms of this zoonotic infection in immunosuppressed patients, have a more severe and chronic course; therefore, the implementation of intensive antibiotic eradication is required [2, 6, 7, 8]. It has been sufficiently proved that bartonellae show significant tropism towards human vascular endothelium which promotes the process of cell adhesion and invasion [9]. The unique pathogenic strategy of *B. henselae* in patients with disturbed immunological status is involved with inducing new blood vessel formation and endothelial cystic disease in the liver and spleen [4, 10, 11]. Furthermore, it has been evidenced that human monocytes, endothelial cells and hepatocytes upregulate the biosynthesis of interleukin-8 (IL-8) during *B. henselae* infection [12]. The chemokine IL-8 functions as a molecular enhancer that stimulates endothelial cell survival and proliferation, and increases production of the matrix metalloproteinases (MMP). Evolutionary benefits of these pathogen-triggered modifications of the host (i.e. induced tumour lesions, neoangiogenesis) are associated with consequential increase in exploitation of the infected habitat [2, 4, 13]. Pathogenicity of *B. henselae* is combined with the ability to survive in infected erythrocytes of different hosts that secondarily creates the possibility of pathogen transmission via blood-sucking arthropods [14, 15, 16]. Experiments conducted by Cotté *et al*. demonstrated that *B. henselae* is able to infect *Ixodes ricinus* ticks and proliferate in their salivary glands. In addition, the application of an artificial membrane-feeding technique confirmed transmission of the analysed pathogen from ticks into blood [17]. Interestingly, the presence of *B. henselae* DNA within the common tick

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individuals removed from humans has been documented (Belluno province, Italy) [18]. On the other hand, Angelakis *et al*. evidenced human cases of SENLAT syndrome (scalp eschar and neck lymphadenopathy after tick bite) caused by *B. henselae* [19]. Furthermore, there are some molecular and serological surveys reporting the clinical co-infection of *B. henselae* and *Borrelia burgdorferi* sensu lato in humans [20, 21]. Despite the case reports evidencing this newly described tick-borne disease complex and an increasing number of published data regarding the presence of *Bartonella* spp. DNA in ticks populations throughout the world [17, 22, 23, 24, 25, 26, 27, 28, 29], molecular studies confirming the co-existence of *B. henselae* and *B. burgdorferi* s.l. in Ixodid ticks have been very limited [27, 30]. In this context, there is a substantial need to assess whether *I. ricinus* ticks collected in Poland may be co-infected by these microorganisms.

Detection of tick-borne pathogens with the application of advanced modifications of the basic PCR technique with consequent automatic sequencing of specific DNA amplicons has become a sensitive and reliable molecular tool in biomedical investigations nowadays. The primary purpose of performed molecular survey was to elucidate the simultaneous occurrence of *B. henselae* and *Borrelia burgdorferi* s.l. DNA in host-seeking individuals of *I. ricinus* ticks representing different developmental stages (nymphs, adult females and males) that were collected from various habitats in central and eastern regions of Poland. The study was designed to determine the potential risk for acquiring human bartonellosis and Lyme borreliosis from questing ticks inhabiting different ecosystems (urban recreational green areas, suburban woods and rural forests) characterizing with variable degrees of anthropogenic influence. Therefore, it has been hypothesized that *I. ricinus* populations occurring in various types of habitats may differ in prevalence levels of *B. henselae* and *B. burgdorferi* s.l. in single and mixed infections. Evaluation of this hypothesis has been conducted in three subsequent stages:

1) molecular identification of investigated human pathogens in analysed samples;
2) determination the prevalence rates of tested microorganisms in *I. ricinus* populations;
3) assessment the frequency of *B. henselae* and *B. burgdorferi* s.l. in ticks living within various ecosystems in the central and eastern parts of Poland.

MATERIALS AND METHODS

Study area and tick sampling procedure. Nymphal and adult individuals of *I. ricinus* ticks were collected during spring in 2008-2009. Questing ticks were sampled by dragging a white woollen flag (1.0 m^2) over lower vegetation at 17 localities representing diverse types of ecosystems throughout the central and eastern regions of Poland. The list of sampling sites comprised urban recreational green areas (city parks and squares) located in Płock, Warsaw, Siedlce, Biała Podlaska and Międzyrzec Podlaski, suburban woods of these towns, and rural forests of Płock, Warsaw and Biała Podlaska Counties. Additionally, forest areas in Ceranów, Jerzyska, Korczew-Mogielnica and Sterdyń within the Nadbużański Landscape Park were also investigated. Collected tick samples were placed into plastic vials filled with 70% ethanol and stored at 4°C. Taxonomic identity of tick samples was confirmed morphologically.

Isolation of gDNA. Tick specimens were rinsed with sterile deionized water. The procedure of genomic DNA extraction from tested ticks was performed with the application of Genomic Mini kit (A&A Biotechnology, Gdynia, Poland), according to the protocol instructions. Nymphal *I. ricinus* ticks were analysed in pools of 5 individuals, whereas adult ticks were processed individually. The quantification of DNA samples was conducted using a NanoVue spectrophotometer (GE Healthcare). Additionally, $A_{260/280}$ and $A_{260/230}$ ratios were calculated to evaluate the sample integrity and contamination of proteins or other organic substances. DNA preparates of high integrity and purity were accepted for further molecular investigations.

Molecular screening of *B. henselae*. Detection of *B. henselae* was based on utilizing a single-step PCR analysis of the citrate synthase (*gltA*) gene, according to the method described by Norman *et al*. [31]. A fragment of the *gltA* gene (approximately 380 bp) was amplified using the oligonucleotide primers: BhCS.781p and BhCS.1137n (Tab. 1). The following thermal cycling conditions were applied: preliminary denaturation at 95°C for 5 min., followed by 40 cycles: at 95°C (1 min.), 55°C (1 min.) and 72°C (2 min.), and subsequently the final elongation at 72°C for 5 min.

PCR detection of *B. burgdorferi* s.l. The presence of *B. burgdorferi* s.l. DNA was confirmed using a conventional PCR technique. It was applied to 2 sets of primers: Fla1/Fla2 [32] and OA149/OA319 [33] in order to amplify fragments of the targeted genes: *fla* and *ospA* (Tab. 1). The length of the PCR amplicons were: 482 and 170 bp, respectively. Each round of PCR reactions included the positive control (DNA of the analysed microorganisms) and the negative control (sterile deionized water).

Table 1. Primers used in the performed molecular studies

Detected pathogen	Amplified gene	Amplicon size (bp)	Primer			Reference
			Name	Type	Sequence (5′-3′)	
B. burgdorferi sensu lato	*fla*	482	Fla1	F	AGAGCAACTTACAGACGAAATTAAT	[32]
			Fla2	R	CAAGTCTATTTTGGAAAGCACCTAA	
	ospA	170	OA149	F	TTATGAAAAAATATTTATTGGGAAT	[33]
			OA319	R	CTTTAAGCTCAAGCTTGTCTACTGT	
B. henselae	*gltA*	380	BhCS.781p	F	GGGGACCAGCTCATGGTGG	[31]
			BhCS.1137n	R	AATGCAAAAAGAACAGTAAACA	

F – forward; R – reverse; bp – base pair.

Visualisation of PCR products. Separation of the specific DNA amplicons was performed using a horizontal gel electrophoresis (2% agarose) under standard conditions. DNA fragments were detected using ethidium bromide (BrEt) staining and UV transillumination. The molecular mass of the PCR products of targeted genes was estimated using DNA Molecular Weight Markers 100-500 bp (DNA-Gdańsk II, Poland).

Nucleotide sequence analysis. The obtained DNA amplicons were purified with the application of Montage™ PCR Centrifugal Filter Devices (Millipore). Sequencing reactions were carried out using the BigDYE Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems), whereas the nucleotide terminators were removed using an ExTerminator kit (A&A Biotechnology, Gdynia, Poland). Both DNA strands were subjected to direct cycle sequencing with the use of an automatic 3130xl Genetic Analyzer (Applied Biosystems). The final stage of the conducted diagnostic procedures was the molecular identification of examined pathogens. This was performed by comparing the results of DNA sequencing with the published sequences in the GenBank® database using the Basic Local Alignment Search Tool (BLASTn), available at the National Center for Biotechnology Information (Bethesda, Maryland, USA).

Statistical analysis. Significance of the differences in prevalence levels and co-infection rates between experimental groups of *I. ricinus* ticks inhabiting investigated habitats was statistically analysed by means of a chi-square test. All computations were carried out using STATISTICA 9.0 software (StatSoft Poland).

RESULTS

Abundance of *I. ricinus* ticks within investigated habitats. A total of 1,571 host-seeking ticks were gathered at 17 locations situated throughout the central and eastern regions of Poland (Tab. 2-3). 865 (55.1%) nymphal individuals of *I. ricinus*, 377 females (24.0%) and 329 males (20.9%) were collected. It should be noted that the common tick populations were found in all types of analysed ecosystems (urban recreational green areas – municipal parks and squares, suburban woods and rural forests). The highest number of ticks was collected from rural forest areas located within Warsaw County (n=213), slightly lower in Biała Podlaska County (n=186) and Płock County (n=165). A minor abundance of *I. ricinus* individuals was recorded in the suburban woods of Warsaw (n=136), while the number of ticks sampled in the forests of the Nadbużański Landscape Park ranged from 42 (Ceranów) – 133 (Korczew-Mogielnica). The lowest size of tick population was ascertained in municipal parks and squares located in Międzyrzec Podlaski (n=21) and Biała Podlaska (n=26).

Molecular survey of *B. henselae* and *B. burgdorferi* s.l. co-existence in *I. ricinus* samples. Pathogen prevalence rates were estimated using the conventional PCR technique with subsequent DNA sequencing of the obtained amplicons. Among the collected ticks, the simultaneous presence of the analysed pathogens was confirmed in 22 samples (1.4%). It should be stressed that dual infection with *B. henselae* and *B. burgdorferi* s.l. was detected only in adult individuals of *I. ricinus* ticks (Tab. 2). The co-infection rate of these tick-borne pathogens in females (4.0%, 15/377) was significantly higher ($p<0.01$) than the prevalence levels calculated for males (2.1%, 7/329). It should be noted that the co-occurrence of analysed microorganisms was confirmed in ticks collected from six localities situated in the central region and one location in the eastern part of Poland (woodlands in Korczew-Mogielnica, rural woodland sites of Warsaw and Płock Counties, urban recreational green areas in Warsaw, and suburban woods of Warsaw, Płock and Biała Podlaska). Furthermore, the prevalence of co-infection in ticks varied depending on the collection site, and ranged from 0.6% (1/165) in rural woods situated in Płock County to 4.4% (6/136) in suburban woods of Warsaw. The intermediate level of mixed infection was found in ticks gathered from the suburban woods of Płock (1.6%, 1/64) and Biała Podlaska (1.7%, 1/60) (Tab. 3).

Table 2. Infection rates of analysed tick-borne pathogens in collected *I. ricinus* specimens (central and eastern parts of Poland 2008-2009)

Developmental stage of ticks	No. of collected ticks	No. (%) of infected ticks		No. (%) of co-infected (*B.h.*+*B.b.*s.l.) ticks
		B.h.	*B.b.*s.l.	
Females	377	45 (11.9)	89 (23.6)	15 (4.0)
Males	329	31 (9.4)	57 (17.3)	7 (2.1)
Nymphs	865	0 (0.0)	48 (5.5)	0 (0.0)
Total	1,571	76 (4.8)	194 (12.3)	22 (1.4)

B.h. – *Bartonella henselae*; *B.b.*s.l. – *Borrelia burgdorferi* sensu lato.

Table 3. Prevalence of investigated microorganisms in *I. ricinus* ticks collected from diverse types of ecosystems in central and eastern regions of Poland 2008-2009.

Sampling area	Habitat type	No. of collected ticks	No. (%) of infected ticks		No. (%) of co-infected (*B.h.*+*B.b.*s.l.) ticks
			B.h.	*B.b.*s.l.	
Ceranów*	w	42	0 (0.0)	4 (6.5)	0 (0.0)
Jerzyska*	w	89	0 (0.0)	3 (3.4)	0 (0.0)
Korczew--Mogielnica*	w	133	2 (1.5)	15 (11.2)	1 (0.7)
Sterdyń*	w	115	1 (0.9)	7 (6.1)	0 (0.0)
Płock County	rw	165	10 (6.1)	18 (10.9)	1 (0.6)
Warsaw County	rw	213	19 (8.9)	37 (17.4)	8 (3.8)
Biała Podlaska County	rw	186	3 (1.6)	16 (8.6)	0 (0.0)
Płock	uga	49	4 (8.2)	7 (14.1)	0 (0.0)
	sf	64	6 (9.4)	9 (14.3)	1 (1.6)
Warsaw	uga	121	9 (7.4)	22 (18.2)	4 (3.3)
	sf	136	13 (9.6)	32 (23.5)	6 (4.4)
Siedlce	uga	45	0 (0.0)	2 (4.4)	0 (0.0)
	sf	67	3 (4.5)	8 (11.9)	0 (0.0)
Biała Podlaska	uga	26	0 (0.0)	3 (11.5)	0 (0.0)
	sf	60	5 (8.3)	6 (10.0)	1 (1.7)
Międzyrzec Podlaski	uga	21	0 (0.0)	1 (4.8)	0 (0.0)
	sf	39	1 (2.6)	4 (10.3)	0 (0.0)
Total		1,571	76 (4.8)	194 (12.3)	22 (1.4)

* Nadbużański Landscape Park; *B.h.* – *Bartonella henselae*; *B.b.*s.l. – *Borrelia burgdorferi* sensu lato; w – woodlands; rw – rural woodlands; uga – urban green areas (city parks and squares); sf – suburban forests.

Molecular screening of *B. henselae* infection in examined *I. ricinus* ticks. The PCR amplification of a 380 bp fragment of the *gltA* gene revealed that 4.8% (n=76) of all collected

I. ricinus ticks were infected with *B. henselae* (Tab. 2). It should be emphasized that there were no *B. henselae*-positive samples in the tested group of nymphal individuals. Furthermore, the occurrence of detected pathogen among the investigated adult ticks was confirmed in the case of 45 females (11.9%) and 31 males (9.4%). Additionally, the significance of differences in the prevalence level between females and males was statistically proved ($p<0.05$). It was shown that ticks inhabiting the suburban woods of Warsaw and Płock characterized the highest prevalence of *B. henselae* (9.6 and 9.4%, respectively) (Tab. 3). Conversely, the lowest infection rate was ascertained in questing ticks collected from woodlands in Sterdyń (0.9%), Korczew-Mogielnica (1.5%) and rural forests of Biała Podlaska County (1.6%). However, the presence of *B. henselae* was not detected in ticks collected at five localities (Ceranów and Jerzyska – the Nadbużański Landscape Park, urban green areas in Siedlce, Biała Podlaska and Międzyrzec Podlaski).

PCR detection of *B. burgdorferi* s.l. in tested *I. ricinus* populations. A total of 194 of the examined ticks (12.3%) were found to be infected with *B. burgdorferi* s.l. (Tab. 2). The highest infection rate of these spirochetes was recorded in females (23.6%, 89/377), a moderate value was estimated in the group of males (17.3%, 57/329), while the lowest prevalence was ascertained in nymphs (5.5%, 48/865). Furthermore, statistical analysis proved the significance of differences in the infection rate of *B. burgdorferi* s.l. between the examined developmental stages of the common tick individuals ($p<0.01$). It is important to note that the analysed spirochetes DNA was detected in ticks collected from all the tested sampling sites throughout the central and eastern parts of Poland (Tab. 3). It was revealed that most *Borrelia*-positive samples were identified in the group of ticks gathered in the suburban woods of Warsaw (23.5%), whereas the lowest prevalence levels of *B. burgdorferi* s.l. were noted in forests off Jerzyska (3.4%) and urban green areas in Siedlce (4.4%) and Międzyrzec Podlaski (4.8%).

Molecular identification of analysed tick-borne pathogens. Species confirmation of the investigated microorganisms was based on direct automatic cycle sequencing of the targeted genes. Four amplicons of the citrate synthase gene of *B. henselae* and five PCR products of the flagellin gene of *B. burgdorferi* s.l. were subjected to sequence analysis. The obtained nucleotide sequences showed 99-100% homology with *B. henselae,* and 100% identity with *B. burgdorferi* s.l. DNA sequences published previously in the GenBank® database.

DISCUSSION

In recent years, considerable alternations have been observed in the geographical range and a remarkable increase in abundance of *I. ricinus* ticks in Europe. Many researchers underline that the common tick distribution and size of its populations may be additionally augmented if trends in global climate changes will continue [34, 35, 36, 37]. This ectoparasite species is a well-documented vector of *B. burgdorferi* s.l. (the etiological agent of Lyme disease), *Anaplasma phagocytophilum* (HGA, human granulocytic anaplasmosis), *Babesia* spp. (human babesiosis) and tick-borne encephalitis virus (TBE, tick-borne encephalitis) [38, 39, 40]. During the last decade, numerous epidemiological studies have been published that indicate an upsurge in the incidence of *I. ricinus*-borne diseases in many European countries [41, 42, 43, 44]. Furthermore, the rapid progress in the development of advanced genetic techniques used in molecular diagnostics of tick-borne diseases (TBD) has led to a marked increase in the number of newly recognized pathogens circulating in *I. ricinus* individuals collected from various ecosystems [42, 43, 45]. In the context of public health, awareness of concomitant human diseases acquired after a single tick bite has risen recently. The clinical implications of tick-borne polymicrobial infections may be involved with significant modifications in the course of these diseases that secondarily increases the probability of misdiagnosis. On the other hand, selection of the most appropriate and successful strategy in antibiotic treatment of possible patterns of human tick-transmitted co-infections has emerged as a serious therapeutic problem [42, 43, 44, 46, 47]. Interestingly, Swanson *et al*. claim that clinicians should take into consideration the likelihood of co-infection when a human tick-borne disease is being diagnosed [47]. Despite many authors emphasizing the necessity of conducting comprehensive molecular surveys evaluating the co-infection rates of diverse spectrum of pathogens in tick populations throughout Europe, there is a scarcity of papers evidencing the co-circulation of microorganisms in the developmental stages of *I. ricinus* ticks occurring in various habitats [39, 48, 49, 50].

In the presented study, the co-existence of *B. henselae* and *B. burgdorferi* s.l. DNA was detected in 22 adults of the tested ticks (1.4%). To the best of our knowledge, this is the first report evidencing the concurrent presence of these targeted pathogens in the common tick individuals collected in Poland. The prevalence of dual infection with analysed microorganisms in host-seeking *I. ricinus* females was approx. 2-fold higher in comparison with the co-infection rate in males. The absence of mixed infection in the examined pools of nymphal individuals was noted. Furthermore, the coincidence of tested pathogens was confirmed in tick specimens gathered from 7/17 sampling sites representing all types of investigated ecosystems. The lowest prevalence of co-infection was recorded in ticks inhabiting the rural forests in Płock County (0.6%) and forested areas in Korczew-Mogielnica (0.7%) located within the Nadbużański Landscape Park. The moderate prevalence of dual infection was ascertained in ticks collected from parks and squares in Płock (1.6%) and Biała Podlaska (1.7%). It is noteworthy that the highest frequency of co-infected ticks was found in samples gathered from urban green areas in Warsaw (4.4%) and rural forests of Warsaw County (3.8%). The higher levels of co-infection rate in ticks inhabiting different collection sites within Warsaw County, in comparison with the other localities, may be influenced by miscellaneous environmental variables, such as larger areas of municipal parks, squares and suburban green areas, higher degree of anthropogenic influence, rapid circulation of pathogenic microorganisms between the vector and its hosts, and the increased abundance of the tick population and host availability. There is a limited number of experimental data confirming the co-existence with investigated pathogens in *Ixodes* spp. populations in Europe. A molecular survey performed by Halos *et al*. revealed that the simultaneous occurrence of *Bartonella* spp. and *B. burgdorferi* s.l. DNA was detected only in 1.0% (1/92)

of questing *I. ricinus* ticks collected from two neighbouring pastures in northern France (Lille area) [51]. Mietze *et al.* using a quantitative real-time PCR analysis obtained similar results. According to these authors, 1.7% (4/230) of *I. ricinus* ticks removed from humans in Germany were co-infected with *B. henselae* and *B. burgdorferi* s.l. [30]. It is noteworthy that both groups of researchers proved the simultaneous presence of analysed tick-borne pathogens in nymphal individuals of the common tick. By contrast, our results did not confirm the co-occurrence of tested microorganisms in this developmental stage of ticks. Additionally, analyses conducted by Holden *et al.* have shown that 1.19% (2/168) of *I. pacificus* ticks collected from Santa Cruz County (California, USA) were co-infected with these pathogenic microorganisms [52]. Specific patterns of multiple infections with different tick-borne pathogens are associated with a wide range of diverse variables. One of the most important factors is the specificity of the interrelationship (antagonistic, neutral or positive) between pathogens co-existing in tick individuals. The complex character of these reciprocal interactions may determine the level of co-infection rates and influence the potential of co-transmission to vertebral hosts [53]. Furthermore, the co-infection prevalences in *I. ricinus* ticks may significantly differ between the sampling areas, depending on the density of tick populations, specific microclimate conditions affecting the development and survival of ticks, number of collected tick specimens, and application of specific methods of pathogen identification [27, 48, 49, 54].

The performed analyses provided molecular evidence supporting the hypothesis regarding the possible involvement of *I. ricinus* in the transmission of *B. henselae* to humans. Although it has been well-established that the common tick may harbor *B. henselae* DNA [18, 30, 55], the biological role of this ectoparasite and other hematophageous arthropods in the life cycle of bartonellae still remains a matter of debate [13, 56, 57, 58]. However, despite the quite low co-infection rate with *B. henselae* and *B. burgdorferi* s.l. in the common tick populations in Poland, the risk of simultaneously acquiring these pathogens by humans during recreational and occupational activities should be taken into consideration. It should be underlined that immunocompromised individuals (organ transplant recipients, HIV-infected and cancer patients, the homeless, drug users and alcoholics, etc.) represent a group of people particularly exposed to risk of contracting a single and mixed tick-borne disease. Consequently, the distribution of various polymicrobial infections in *I. ricinus* populations inhabiting different ecosystems should be further carefully monitored to evaluate the potential implications of a single tick bite for human health. In this context, a comprehensive analysis of the diverse environmental factors affecting human exposure to co-infected ticks, and the drawing of maps of designating areas characterized by a high prevalence of single and mixed infections in ticks may be helpful in formulating more effective strategies to prevent tick-transmitted diseases.

CONCLUSIONS

This is the first report providing molecular evidence of concurrent presence of *B. henselae* and *B. burgdorferi* s.l. DNA in *I. ricinus* ticks collected in Poland. The presented results strengthen the hypothesis that the common tick may be involved in the circulation of *B. henselae* within different ecosystems in Poland. Despite the low co-infection status of investigated pathogens in examined individuals of the common tick, the possibility of simultaneously acquiring of Lyme disease and bartonellosis should be considered. Therefore, more detailed molecular and serological studies are required to reveal the multi-level factors influencing the risk of potential human co-infections after a single tick bite. On the other hand, further investigations regarding the dispersal of co-infected ticks in various habitat types in Poland are highly recommended.

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