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Diversity of *Borrelia* spirochetes and other zoonotic agents in ticks from Kyiv, Ukraine

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ABSTRACT

Lyme borreliosis (LB) is caused by tick-borne spirochetes of the *Borrelia burgdorferi* sensu lato complex. LB is the most prevalent vector-borne illness in Ukraine, but current data on the prevalence of LB pathogens in their tick vector, *Ixodes ricinus,* are lacking. *I. ricinus* ticks may also carry *Borrelia miyamotoi*, an emerging relapsing fever group spirochete that has been implicated in human illness. Despite its zoonotic potential, the prevalence of *B. miyamotoi* in ticks has not been examined in Ukraine. Similarly, data on the prevalence of other important tick-borne pathogens, *Anaplasma phagocytophilum*, *Babesia* spp., *Bartonella* spp., *Francisella tularensis*, and *Rickettsia* spp., in ixodid ticks are scarce or even absent. Thus, the overall objective of this study was to investigate the prevalence of these tick-borne pathogens in questing *I. ricinus* and *Dermacentor reticulatus* ticks collected in recreational parks of Kyiv, the most densely populated city of Ukraine. A total of 182 adult *I. ricinus*, 98 nymphal *I. ricinus*, and 98 adult *D. reticulatus* ticks were molecularly analyzed for the presence of these pathogens. As a result, the study shows a greater diversity of *Borrelia* genospecies in questing *I. ricinus* ticks than previously reported. The most prevalent genospecies in adult *I. ricinus* ticks were *B. afzelii* (7.7%), followed by *B. burgdorferi* sensu stricto (s.s.) (2.2%) and *B. garinii* (0.5%). In contrast, *B. burgdorferi* s.s. was most dominant in unfed *I. ricinus* nymphs (67.3%). Moreover, *B. afzelii* was detected in 11.2% of nymphs, but only 1.0% of nymphal ticks were positive for *B. garinii* and *B. valaisiana*. Importantly, this study provides the first record of *B. miyamotoi* detected in *I. ricinus* ticks from Ukraine (1.1%). Furthermore, the report is also the first to document other vector-borne pathogens, *Bartonella henselae*, *Rickettsia conorii*, and *Rickettsia mendelii,* in ixodid ticks from Ukraine. In summary, this work offers the latest data on the diversity and prevalence of the important zoonotic tick-borne agents in questing ticks from Kyiv, Ukraine. The data will help to better gauge the risk associated with vector-borne infections to which residents and guests of Ukraine's capital may be exposed.

1. Introduction

Ticks are vectors that harbor various pathogens of human and animal diseases (Brites-Neto et al., 2015; Day, 2011). Of medical importance are spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex. *B. burgdorferi* s.l. causes Lyme borreliosis (LB), the most prevalent arthropod-borne disease in the temperate regions of the northern hemisphere (Rizzoli et al., 2011). Due to its expanding geographical distribution, LB is becoming an increasingly relevant health risk (Rizzoli et al., 2011).

The *B. burgdorferi* s.l. complex comprises at least 20 genospecies (Stanek et al., 2011). In Europe, the main pathogenic genospecies are *B. afzelii*, *B. garinii*, and *B. burgdorferi* sensu stricto (s.s.), which are transmitted by the ixodid tick, *Ixodes ricinus*. *B. afzelii* and *B. garinii* are most often associated with acrodermititis chronica atrophicans and neuroborreliosis, respectively. *B. burgdorferi* s.s. predominantly causes arthritis and neuroborreliosis (Stanek et al., 2011). LB is a major public health problem in Europe: approximately 65,500–85,000 LB cases are estimated to occur each year (Hubálek, 2009; Lindgren and Jaenson, 2006; van den Wijngaard et al., 2017).

In Central and Eastern Europe, the LB incidence may range from 1.0 to 9.1 (Belarus), 16.4 (Slovakia) and 25.0–35.0 (Lithuania) to 4.8–106.8 (Poland) cases per 100,000 individuals per year (Bochnicková et al., 2012; Lindgren and Jaenson, 2006; Paradowska-Stankiewicz and Chrześcijańska, 2016; Reye et al., 2013). In Ukraine, LB is also the most prevalent vector-borne illness that commonly manifests itself as erythrema migrans (64.9% cases) and neuroborreliosis (21.4%) (Biletska et al., 2011). In the period of 2000–2010, 4597

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cases were officially reported in Ukraine. According to the Ministry of Health of Ukraine, 3413 and 2758 cases were recorded in 2015 and 2016, respectively (Nebogatkin et al., 2017). LB cases have been registered in all the administrative regions of Ukraine including the Autonomous Republic of Crimea (Biletska et al., 2011). In 2016, the highest LB incidences rates (number of cases per 100,000 individuals per year) were recorded in Cherkasy (13.86), Kyiv (11.84), Sumy (11.5), Chernihiv (10.6), Ternopil (10.5), Vinnytsya (10.48) regions, and in the city of Kyiv (22.73) (Nebogatkin et al., 2017). Though most new LB cases are reported in May through September (81.7–85.6%), human LB may occasionally occur in April, October, and November.

Studies on the prevalence of *Borrelia* among *I. ricinus* ticks in Ukraine are very limited. The most comprehensive study involved 2811 adult *I. ricinus* that were collected across twelve regions of Ukraine over 2003–2006. The ticks were examined by dark-field microscopy for the presence of *Borrelia* spirochetes (Biletska et al., 2008). On average, 9.5% *I. ricinus* ticks were positive for *Borrelia*. The prevalence of *B. burgdorferi* s.l. varied from 5.6% in the Carpathians to 10.8% in Polissya, and reached up to 25.0% in some biotopes (Biletska et al., 2008). The most recent study showed that the prevalence of *B. burgdorferi* s.l. in *I. ricinus* ticks collected in Kyiv over 2013–2014 was 4.0% (Didyk et al., 2017). In that study, only two genospecies, *B. afzelii* and *B. garinii*, were identified, whereas *B. burgdorferi* s.s. was not detected in any of 696 *I. ricinus* ticks tested (Didyk et al., 2017).

In addition to LB pathogens, *I. ricinus* may also carry *Borrelia miyamotoi*, an emerging relapsing fever group spirochete that has been implicated in human disease (Sato et al., 2014). In 2011, the first human cases of *B. miyamotoi* infections were reported in Russia, where 1–16% of *I. persulcatus* ticks were found infected with *B. miyamotoi* (Platonov et al., 2011). In the United States, *B. miyamotoi* infections are prevalent in LB endemic areas and the prevalence may reach up to 15.4% in *Ixodes* ticks (Crowder et al., 2014; Krause et al., 2013). However, despite its zoonotic potential, the prevalence of *B. miyamotoi* in ticks has not been examined in Ukraine to date.

In Central Europe, *Dermacentor reticulatus* is the second most reported ixodid tick species after *I. ricinus* (Rubel et al., 2014). *D. reticulatus* is considered the main vector of *Babesia canis*, *Babesia caballi*, *Rickettsia slovaca*, and *Rickettsia raoultii* (Rubel et al., 2016). Moreover, adult questing *D. reticulatus* may carry *Anaplasma phagocytophilum*, *Bartonella henselae*, and *F. tularensis*, although its vector competence for these pathogens is not yet proven (Rubel et al., 2016).

The bacterial and protozoal pathogens that ixodid ticks often harbor are of public health significance (Brites-Neto et al., 2015). *A. phagocytophilum* may cause severe disease in several mammalian species (Stuen et al., 2013) and is an emerging pathogen responsible for human granulocytic anaplasmosis (Bakken and Dumler, 2015; Jin et al., 2012).

Francisella tularensis is a bacterial agent of tularemia that exists in enzootic cycles across the Northern Hemisphere. Humans may become exposed to *F. tularensis* via arthropod bites, inhalation of aerosolized bacteria, or ingestion of contaminated products of animal origin (Staples et al., 2006). *I. ricinus* ticks are considered to be significant vectors in the ecology of this disease (Gyuranecz et al., 2011).

Bartonella spp. can establish persistent bloodstream infections in humans and animals (Day, 2011; Florin et al., 2008; Nelson et al., 2017). Bartonellosis may become particularly significant in immunocompromised individuals (e.g., HIV-infected individuals with acquired immunodeficiency syndrome) (Day, 2011). Although cat fleas are the primary vectors for *B. henselae*, transmission by ticks has been previously suggested (Angelakis et al., 2010).

The spotted fever group (SFG) rickettsioses are infections caused by established and emerging rickettsial agents (Parola et al., 2013). *Rickettsia* spp. are transmitted to humans via arthropods and may result in potentially fatal diseases (Wood and Artsob, 2012).

In addition to bacterial agents, ixodid ticks may also transmit pathogenic protozoa. Though *Babesia microti* and *Ba. divergens* are known to cause human babesiosis, several other *Babesia* species have been implicated in human disease (Colwell et al., 2011; Hunfeld et al., 2008). The clinical manifestations of this emerging infectious disease may range from subclinical infection to a fulminant and fatal disease (Vannier and Krause, 2012).

Despite the medical significance of the above-mentioned pathogens, data on their prevalence in ixodid ticks from Ukraine are scarce. Thus, the overall objective of this study was to investigate the prevalence of *A. phagocytophilum*, *Babesia* spp., *Bartonella* spp., *Borrelia* spp., *F. tularensis*, and *Rickettsia* spp. in questing *I. ricinus* and *D. reticulatus* ticks collected in Kyiv, the most densely populated city of Ukraine.

2. Materials and methods

2.1. Origin of ticks and DNA extraction

Adult *I. ricinus* (n = 182), nymphal *I. ricinus* (98), and adult *D. reticulatus* (98) ticks collected during the month of May 2016 within and immediately outside the administrative limits of Kyiv, Ukraine were used for this study. Tick collection was performed at nine sites: Dorogozhychi Park (sampling area 1), Druzhby Narodiv Park (sampling area 2), Feofaniya Park (sampling area 3), Holosiivskyi Forest (sampling area 4), Hryshko National Botanical Garden (sampling area 5), the Kyiv Zoo (sampling area 6), and Boyarka (sampling area 7). A map of the sampling sites and indices of abundance can be found elsewhere (Rogovskyy et al., 2017). Individual ticks were flash frozen in liquid nitrogen and crushed with disposable plastic pestles (VWR International, LLC, USA). DNA was extracted from adult and nymphal ticks utilizing the DNeasy Minikit and QIAamp DNA Mini Kit (Qiagen Inc., USA) according to the manufacturer's protocols with the following modification. Buffer AL was added to the suspension, which was then vortexed and incubated at 70 °C for 16 h. Extracted DNA was stored at −20 °C until use.

2.2. 23S rRNA, ospA, and B. miyamotoi intergenic spacer regions (IGS) real-time PCR

DNA samples of adult *I. ricinus* (n = 182) were screened by 23S rRNA real-time PCR and PCR-positive DNA was then tested by the confirmatory *ospA* real-time PCR using previously published primers and protocol (Dibernardo et al., 2014). In parallel, 182 DNA samples were analyzed by *B. miyamotoi* IGS real-time PCR followed by the confirmatory *B. miyamotoi glpQ* real-time PCR assay as described (Dibernardo et al., 2014; Ullmann et al., 2005). All real-time PCR reaction mixtures were prepared in 2x TaqMan® Universal Mastermix (Life Technologies, USA) and contained 300–600 nM of each primer and 200 nM of probe. Amplification was performed using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, USA). Thermocycling conditions were as follows: activation of AmpErase at 50 °C for 2 min, 10 min at 95 °C for denaturation of AmpErase and activation of AmpliTaq Gold® Polymerase, followed by 40 cycles of amplification with denaturation at 95 °C for 15 s and annealing at 58 °C for 1 min. The analysis of real-time data was carried out via CFX Manager software (Bio-Rad Laboratories, USA). All primers and probes were previously published (Dibernardo et al., 2014). For each PCR run, previously sequenced DNA samples were used as positive controls. Nuclease-free water (Life Technologies, USA) was used as a negative control.

2.3. Borrelia-specific nested PCR

All *ospA*-negative DNA samples of adult *I. ricinus* and DNA samples obtained from 92 nymphs were subjected to *Borrelia*-specific nested PCR, which amplified a 587-bp fragment of the 16S-23S *IGS* (Bunikis et al., 2004; Scott, 2005). Specifically, the master mix contained 0.2 mM each dNTP, 0.5 μM of the forward and reverse primers, 1 unit of Taq DNA Polymerase with ThermoPol® Buffer (New England BioLabs