Prevalence and distribution of *Borrelia burgdorferi sensu lato* genotypes among ixodid ticks in three regions of Ukraine


*Bila Tserkva National Agrarian University, Bila Tserkva, Ukraine
**Battelle Memorial Institute, Columbus, USA

To improve our understanding and to develop strategies to control Lyme borreliosis, this study focused on assessing the prevalence of clinically relevant *Borrelia* genotypes in ixodid ticks collected from different regions of Ukraine. Ixodid ticks were collected from vegetation and animal hosts in Kyiv, Cherkasy, and Mykolaiv regions of Ukraine (2021). The ticks were then tested by polymerase chain reaction (PCR) for the presence of the *B. burgdorferi sensu lato* complex and genotyped using primers for *B. burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii*. In total, 1132 ixodid ticks were examined. In Kyiv region, *Ixodes ricinus* was the most common species (79.7%), in Cherkasy region, *Dermacentor reticulatus* was most common (72.7%), and in Mykolaiv region, *Hyalomma marginatum* was the most common species (76.4%). PCR analysis showed that *I. ricinus* and *D. reticulatus* are the main vectors of the *B. burgdorferi sensu lato* complex, especially in Kyiv and Cherkasy regions, where *I. ricinus* had a significantly higher total *Borrelia* infection rate (29.2%) than *D. reticulatus* (15.9%). In Mykolaiv region, *Borrelia* was not detected. Genotypic analysis revealed a significantly higher prevalence of the *B. afzelii* (15.6%) over the *B. burgdorferi sensu stricto* genotype at 9.3%. The *B. garinii* genotype was not detected in this study. This study analyzes the prevalence of ixodid ticks and genotypes of the Lyme borreliosis pathogen in Northern, Central and Southern Ukraine. In general, the results of the study indicate a widespread presence of *borrelia* in the northern and central regions, while no *Borrelia* were detected in the southern region. In addition, the *B. afzelii* genotype prevailed in Kyiv and Cherkasy regions.

Keywords: ixodid ticks, *Borrelia burgdorferi*, polymerase chain reaction; Sanger sequencing; prevalence.

**Introduction**

Lyme borreliosis (LB) remains one of the most common tick-borne diseases (Goren et al., 2023). *Borrelia* spp. are divided into two distinct groups: one is responsible for LB in humans and animals, known as the *B. burgdorferi sensu lato* complex (*B. burgdorferi* s. I), the other is associated with relapsing fever. The *B. burgdorferi* s. I complex includes approximately 21 genotypes, and the genotypic composition differs geographically (Cutler et al., 2017). In Europe, the most common *Borrelia* genotypes are *B. burgdorferi sensu stricto* (*B. burgdorferi* s. s.), *B. garinii* and *B. afzelii*, while other genotypes: *B. lusitaniae*, *B. valaisiana*, *B. bissettiae*, *B. bavariensis*, and *B. spielmani* have been identified as pathogenic in individual cases of Lyme disease (LD). While *B. japonica*, *B. garinii*, and *B. afzelii* are mostly found in Europe and Asia, *B. valaisiana*, *B. tautomixis*, *B. sinica*, and *B. yangtzensis* are most common in Asia. In contrast, the *B. burgdorferi* s. s. genotype (and other less common genotypes) are mostly responsible for LB in humans and animals in the United States (Masur, 2004; Barbour & Qiu, 2019; Mysterud et al., 2019).

Ixodid ticks are universal ectoparasites of various species of reptiles, birds, and mammals and play a role in the circulation of LB pathogens. The circulation of LB pathogens is closely related to the prevalence of ixodid ticks and the presence of reservoir and hosts susceptible to *B. burgdorferi* (Boulanger et al., 2019). Ticks of the species *Ixodes ricinus* and *Dermacentor reticulatus* are common in most of Ukraine, except for the coast of the Black and Azov Seas, where ticks of the species *Hyalomma marginatum* and sometimes *D. reticulatus* are more common (Akimov & Nebogatkin, 2011a, 2012). Previous studies have shown that the average combined prevalence of *B. burgdorferi* s. I in *I. ricinus* from five Ukrainian cities was 28% (Levitska et al., 2021). In addition, the prevalence of the *B. burgdorferi* s. I complex among *I. ricinus* ticks in the Kyiv region (Northern Ukraine) was 10.4% (Rogowsky et al., 2018). In Zaporizhzhya region (Southeastern Ukraine), the prevalence of *B. burgdorferi* s. I among *I. ricinus* was 32.3% (Kovryha et al., 2021). In Western Ukraine, *B. burgdorferi* was detected in 29.3% of *I. ricinus* and 31.9% of *D. reticulatus* (Ben & Lorysky, 2019).

Approximately 35,000 cases of human LB are reported annually in the United States (Centers for Disease Control and Prevention, 2021). In Europe, this figure is about 85,000 (Lindgren & Jaenson 2006). In Ukraine, more than 2,500 people are diagnosed with LB annually, and in 2022 the number of registered cases increased to almost 4,000 (Rogovskyy et al., 2020). Most studies in Ukraine have focused on the prevalence of the *B. burgdorferi* s. I complex, but there are some reports of specific genotypes such as *B. burgdorferi* s. s., *B. afzelii*, *B. spielmani*, and *B. valaisiana* in ixodid ticks (Rogovskyy et al., 2018; Levitska et al., 2021). Our previous studies in Ukraine have shown that the number of cases of LB in dogs is increasing, and while human LB cases are reported throughout the country, most cases are reported in the Northern and Central regions (Panteleienko et al., 2022). Thus, understanding the prevalence of vector-competent ixodid ticks and clinically relevant genotypes of *B. burgdorferi* spp. is crucial for LB research and the development of One Health strategies for tick-borne disease control and prevention.

The aim of the study was to evaluate the distribution of *I. ricinus*, *D. reticulatus* and *H. marginatum* tick populations collected in the Northern, Central and Southern Ukraine and to determine the prevalence of clinically significant genotypes of *B. burgdorferi* s. I among ixodid ticks.
Materials and methods

Tick collection. Questing ticks were collected from April to October 2021 in the Kyiv, Cherkasy, and Mykolaiv regions of Ukraine. Ticks were collected in forest parks and forest plantations, in meadows and animal pastures. We collected questing ticks on sunny days, in the morning and in the evening after the dew had dried. To collect questing ticks, a flag made of fleecy fabric of monochromatic light colors (1 m²) was used. The flag was slowly dragged over the vegetation and inspected every 10 m. The found ticks were placed in plastic tubes for further morphological identification. By prior arrangement, we received engorged ticks from local veterinarians from the study regions during 2021, removed from domestic animals (dogs, cats), farm animals (cows, sheep) and wild animals (wild boar, fallow deer, roe deer). The ticks were fully or partially engorged. The collected ticks were identified to the species level using a graphic guide (Estrada-Pena et al., 2004). All collected tick specimens were stored in 70% ethanol at 5 °C until further PCR studies.

DNA extraction and PCR. Ticks were washed with sterile distilled water and dried on filter paper. The ticks were transferred individually to Eppendorf tubes (1.5 mL) and mechanically crushed with sterile scissors. The collected ticks were identified to the species level using a graphic guide (Estrada-Pena et al., 2004). All collected tick specimens were stored in 70% ethanol at 5 °C until further PCR studies.

Tick pools were formed from 10–12 individual DNA samples of ticks that were collected from 10–12 individual DNA samples of ticks that were collected from wild animals, the remaining 93 were collected from domestic animals, Kyiv region; 16 out of 44 D. reticulatus were collected from wild animals, the remaining 28 from domestic animals, Kyiv region; all H. marginatum were collected from farm animals, Mykolaiv region.

In the Kyiv region (Northern Ukraine), 580 ixodid ticks were collected. The proportion of I. ricinus (462 specimens, 79.7%) was significantly higher (P < 0.0001) than the proportion of D. reticulatus (118 specimens, 20.3%) collected. In the Cherkasy region (Central Ukraine), we observed the opposite trend in the distribution. Of 256 ixodid ticks, the majority were D. reticulatus (186 specimens, 72.7%), while 70 were I. ricinus (27.3%) (P < 0.0001). In Mykolaiv region (Southern Ukraine), the majority of the 296 ixodid ticks were H. marginatum (226 specimens, 76.4%), significantly outnumbering both I. ricinus (22 specimens, 7.4%) and D. reticulatus (48 specimens, 16.2%) in this region (P < 0.0001). The ratio of I. ricinus to D. reticulatus ticks was about 4:1 in the Kyiv region, about 1:3 in the Cherkasy region and about 1:2 in the Mykolaiv region. There is a statistically significant difference in the ratio between the Kyiv and Cherkasy regions (P < 0.0001) and between the Kyiv and Mykolaiv regions (P < 0.0001). However, there is no significant difference in the ratio of I. ricinus to D. reticulatus tick populations between the Cherkasy and Mykolaiv regions (P = 0.5494, Fig. 2).

PCR screening. For B. burgdorferi s. l. PCR analysis of tick DNA pools revealed 24 (n = 208) positive I. ricinus pools, 9 (n = 108) positive D. reticulatus pools, and no positive H. marginatum pools. Of these 396 individual tick DNA samples, 218 were positive for B. burgdorferi s. l. Amplification products were of the expected size (325 bp). PCR products were confirmed by DNA sequencing.

Table 1 shows the statistically significantly higher level of infection with the B. burgdorferi s. l. complex at 29.2%
Infestation of ixodid ticks with B. burgdorferi s. l. complex in three regions of Ukraine

Table 2

<table>
<thead>
<tr>
<th>Origin of ticks</th>
<th>I. ricinus</th>
<th>D. reticulatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyiv: from vegetation</td>
<td>134/339</td>
<td>39.5</td>
</tr>
<tr>
<td>Kyiv: from domestic animals</td>
<td>9/93</td>
<td>10.0</td>
</tr>
<tr>
<td>Kyiv: from wild animals</td>
<td>2/30</td>
<td>0.7</td>
</tr>
<tr>
<td>Kyiv: Combined</td>
<td>145/462</td>
<td>31.4</td>
</tr>
<tr>
<td>Cherkasy: from vegetation</td>
<td>16/54</td>
<td>29.6</td>
</tr>
<tr>
<td>Cherkasy: from domestic animals</td>
<td>1/16</td>
<td>6.3</td>
</tr>
<tr>
<td>Cherkasy: Combined</td>
<td>17/70</td>
<td>24.3</td>
</tr>
<tr>
<td>Mykolaiv: from vegetation</td>
<td>0/17</td>
<td>0.0</td>
</tr>
<tr>
<td>Mykolaiv: from domestic animals</td>
<td>0/5</td>
<td>0.0</td>
</tr>
<tr>
<td>Mykolaiv: Combined</td>
<td>0/22</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>162/554</td>
<td>29.2</td>
</tr>
</tbody>
</table>

PCR screening for genotypes. Of all ticks collected in Kyiv and Cherkasy regions (n = 836), 130 (15.6%) were positive for B. afzelii, significantly higher than the number of ticks positive for B. burgdorferi s. s – 78 (9.3%, P = 0.0004). Of the 218 B. burgdorferi s. l. positive ticks, 35.8% (78) were B. burgdorferi s. s., 59.6% (130) were B. afzelii, and 4.6% (10) were negative for the genotypes tested. No ticks in this study tested positive for B. garinii.

The prevalence of the B. afzelii genotype in I. ricinus ticks (19.2%, 102/532) was significantly different from the prevalence of B. afzelii genotype in D. reticulatus ticks (9.2%, 28/304, P = 0.0001). However, the prevalence of B. burgdorferi s. s. genotype in I. ricinus (10.2%, 54/532) was not statistically different from the prevalence of B. burgdorferi s. s. in D. reticulatus (8.0%, 24/304, P = 0.3233).

Fig. 2. Species composition of ixodid ticks in three regions of Ukraine

The combined prevalence of ixodid ticks with B. burgdorferi s. l. complex collected in the Kyiv region was 27.9% (162/580). Among these, the prevalence in I. ricinus was 31.4% (145/462) and D. reticulatus was 14.4% (17/118). In the Cherkasy region, the combined prevalence in ixodid ticks was 21.9% (56/256). Among these, the prevalence in I. ricinus was 24.3% (17/70) and in D. reticulatus was 21.0% (39/186). There was no significant difference in the prevalence of the B. burgdorferi s. l. complex between ixodid ticks collected from the Kyiv and the Cherkasy regions (P = 0.0727). In the Mykolaiv region, no positive results for B. burgdorferi s. l. DNA were found among any of the D. reticulatus (0/48) or I. ricinus (0/22) ticks (Table 2 and Fig. 3).

Fig. 3. Prevalence of B. burgdorferi s. l. among ixodid ticks in Kyiv and Cherkasy regions, Ukraine

Discussion

To better understand the epidemiology of LB in Ukraine, we collected and screened ixodid ticks for the presence of B. burgdorferi s. l. complex and three important genotypes: B. afzelii, B. burgdorferi s. s., and B. garinii. We found regional differences in tick populations, that questing ixodid ticks were more infected with B. burgdorferi s. l. than engorged ixodid ticks, and that B. afzelii was the most prevalent genotype. We also showed that the prevalence of B. afzelii and B. burgdorferi s. s. genotypes between Kyiv and Cherkasy regions was proportionally similar. This study complements previous studies conducted in Ukraine on the distribution of ixodid ticks and the distribution of B. burgdorferi s. l.

Regional differences in the prevalence of ixodid ticks in Ukraine was consistent with ecological differences between regions. In Kyiv region, I. ricinus ticks were more prevalent (79.7%) than D. reticulatus. This is consistent with previous studies that showed greater prevalence of I. ricinus ticks in Kyiv region (Rogovskyy et al., 2017). In Cherkasy region, D. reticulatus were more prevalent (72.7%) than I. ricinus. While D. reticulatus were shown to be more adapted to the western and northern regions in the early 2000s, their dominance shifted to the central and southern regions of Ukraine (Akimov & Nebogatkin, 2011b; Fedonik et al., 2021). Consistent with other studies, we reveal a significant prevalence of H. marginatum (76.7%) in Mykolaiv region (Akimov & Nebogatkin, 2011a). These observed variations can be explained by the synergy of various environmental factors, including climate, vegetation, hosts, land use practices, and other physical and geographical characteristics of the territories (Medlock et al., 2013; Rubel et al., 2016). Ixodes ricinus preferred forests, forest belts, and city parks, while D. reticulatus preferred meadows, glades, and pastures for animals. Previous studies of the distribution of I. ricinus ticks in Ukraine also indicated a higher prevalence of this tick species in urbanized landscapes compared to natural areas (Akimov & Nebogatkin, 2022).
Variations in the prevalence of vector–competent ixodid ticks affect the prevalence of the *B. burgdorferi* s. l. complex (Estrada-Peña et al., 2018). In Ukraine, studies on the prevalence of *B. burgdorferi* are mainly associated with *I. ricinus* ticks (Didyk et al., 2017; Rogovskyy et al., 2018; Levytska et al., 2021). In the northwestern regions of Ukraine, 26.0% of *I. ricinus* ticks were infected with *B. burgdorferi* s. l. complex (Levytska et al., 2021). In Western Ukraine, 29.0% of *I. ricinus* ticks were infected with *Borrelia* and 32.0% of *D. reticulatus* were infected. Infection of *D. reticulatus* ticks with the *B. burgdorferi* s. l. complex has not been previously reported in Northern or Central Ukraine. In this study on ticks of Northern and Central Ukraine, we found that 30.0% of all collected *I. ricinus* ticks and 16.0% of all collected *D. reticulatus* ticks were positive for *B. burgdorferi* s. l. complex.

*Questing* ixodid ticks had a higher level of infection (29.8%) with the *B. burgdorferi* s. l. complex compared to engorged ixodid ticks (6.7%). This difference may be due to the fact that substances present in mammalian blood can inhibit PCR amplification (Beichel et al., 1996; Michalski et al., 2020). Interestingly, one Ukrainian study showed that infection levels in questing *I. ricinus* ticks (27–44%) were higher than infection levels found in engorged *I. ricinus* ticks (0–14%), and *D. reticulatus* was not tested for the *B. burgdorferi* s. l. complex (Levytska et al., 2021). Differences in the results of these two studies can be explained by a number of factors, including climate, geography, tick habitats, methods, etc., and emphasizes the complex interaction of these dynamics on LD. Larger studies are needed to fully understand all the links in the *B. burgdorferi* s. l. epizootic chain.

None of the *H. marginatum* (n = 226), *D. reticulatus* (n = 36), or *I. ricinus* (n = 22) ticks from the Southwestern region of Mykolaiv were positive for *B. burgdorferi* s. l. This is consistent with previous studies which showed the absence of *B. burgdorferi* s. l. complex in most tick species collected in Southern Ukraine, with the exception of *I. ricinus* (8.6–12.7%) (Kovryha et al., 2021). The absence of *B. burgdorferi* s. l. positive ticks collected in Mykolaiv may reflect a smaller sample size. Climatic features such as high soil and air temperatures, combined with reduced humidity, may also affect the tick species compositions and spread of LB pathogens (Panteleienko et al., 2022).

In this study, the most common borrelia genotype was *B. afzelii* (14.3%). The *B. burgdorferi* s. s. genotype was less common (8.6%). In addition, the prevalence of the *B. afzelii* genotype was significantly higher in *I. ricinus* ticks (18.4%) compared to *D. reticulatus* (8.0%). Regionally, the *B. afzelii* genotype was more prevalent in Kyiv region (17.6%) compared to Cherkasy region (10.9%). The *B. burgdorferi* s. s. genotype had narrower variations among *D. reticulatus* (8.0%) and *I. ricinus* (9.7%) ticks, as well as between Kyiv (9.5%) and the Cherkasy (8.9%) regions. A previous study by Didyk et al. (2017) showed that *I. ricinus* ticks from Kyiv region were highly infected with *B. afzelii* (96.4%), but *B. burgdorferi* s. s. was not detected. Our study confirms the high prevalence of *B. afzelii* but also reveals the presence of *B. burgdorferi* s. s. in Kyiv region. Finally, we found that 4.6% of *B. burgdorferi* s. l. positive ticks (n = 10) were not positive for *B. burgdorferi* s. s., *B. afzelii*, or *B. garinii*. This emphasizes the need for further research to identify other clinically important genotypes in Ukraine.

**Table 3**

<table>
<thead>
<tr>
<th>Origin of ticks</th>
<th><em>I. ricinus</em></th>
<th><em>B. burgdorferi</em> s. s.</th>
<th><em>B. afzelii</em></th>
<th><em>D. reticulatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyiv:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from vegetation</td>
<td>39/339</td>
<td>11.5</td>
<td>90/339</td>
<td>26.5</td>
</tr>
<tr>
<td>from animals</td>
<td>8/123</td>
<td>6.5</td>
<td>3/123</td>
<td>2.4</td>
</tr>
<tr>
<td>Combined</td>
<td>47/462</td>
<td>10.2</td>
<td>93/462</td>
<td>20.1</td>
</tr>
<tr>
<td>Cherkasy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from vegetation</td>
<td>6/54</td>
<td>11.1</td>
<td>9/54</td>
<td>16.7</td>
</tr>
<tr>
<td>from animals</td>
<td>1/66</td>
<td>0.6</td>
<td>0/61</td>
<td>0.0</td>
</tr>
<tr>
<td>Combined</td>
<td>7/70</td>
<td>10.0</td>
<td>9/70</td>
<td>13.0</td>
</tr>
<tr>
<td>Total</td>
<td>54/532</td>
<td>10.2</td>
<td>102/532</td>
<td>19.2</td>
</tr>
</tbody>
</table>

Note: * the indicated values correspond to: PCR-positive ticks / total number of ticks tested by PCR.

**Conclusion**

This study adds to the knowledge of ixodid tick populations and Borrelia genotypes in Northern, Central and Southern Ukraine. Both *D. reticulatus* and *I. ricinus* are important vectors and *B. afzelii* and *B. burgdorferi* s. s. were the most common genotypes. Understanding the dynamics of LD is crucial for effective disease management and prevention strategies. Future research should focus on elucidating the environmental and natural factors that influence tick populations and clinically relevant Borrelia genotypes in Ukraine. Comprehensive studies combining ecological, climatic, and molecular analyses will provide valuable insights into the complex interactions that contribute to the spread of LB and help develop targeted interventions to mitigate its impact on human and animal health.

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**References**


