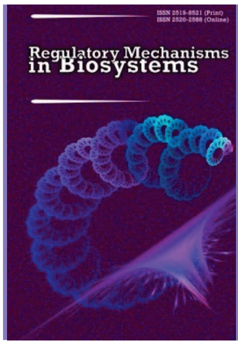

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Prevalence and distribution of *Borrelia burgdorferi sensu lato* genotypes among ixodid ticks in three regions of Ukraine

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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. l.*), the other is associated with relapsing fever. The *B. burgdorferi s. l.* 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. spielmanii* 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. tanukii*, *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 (Masuzawa, 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, 2022). Previous studies have shown that the average combined prevalence of *B. burgdoferi s. l.* in *I. ricinus* from five Ukrainian cities was 26% (Levytska et al., 2021). In addition, the prevalence of the *B. burgdorferi s. l.* complex among *I. ricinus* ticks in the Kyiv region (Northern Ukraine) was 10.4% (Rogovskyy et al., 2018). In Zaporizhzhya region (Southeastern Ukraine), the prevalence of *B. burgdorferi s. l.* 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 & Lozynsky, 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. l.* complex, but there are some reports of specific genotypes such as *B. burgdorferi s. s.*, *B. afzelii*, *B. spielmanii*, and *B. valaisiana* in ixodid ticks (Rogovskyy et al., 2018; Levytska 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. l.* 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 m^2) 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 with 200 µL of 0.9% (w/v) sodium chloride solution (YURiA-PHARM, Cherkassy, Ukraine). For DNA extraction, the IndiSpin Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany) was used with some modifications. In particular, tick suspensions were incubated with 25 µL of proteinase K (600 U/mL) at 56 °C for 1.5 hours. DNA was eluted in 150 µL of elution buffer. DNA samples were stored at –20 °C until PCR testing.

Tick pools were formed from 10–12 individual DNA samples of 10 µL each. The pools were tested for the presence of the *B. burgdorferi s. l.* complex (16s rRNA) (Levytska et al., 2021). Then, from the positive pools for the presence of the *B. burgdorferi s. l.* complex, individual tick DNA samples were tested for *B. burgdorferi s. s.* (16s-BB), *B. afzelii* (16s-VS461), and *B. garinii* (16s-BG) genotypes as previously described (Marconi & Garon, 1993).

Reaction composition (25 µL) included 12.5 µL of One Taq Quick-Load 2X Master Mix with standard buffer (New England Biolabs, Ipswich, Massachusetts, USA), 0.5 µL of the respective primer sets F and R (concentration 100 pMol/µL), 8.5 µL of deionized water, and 3 µL of extracted DNA. Nucleic acid amplification was performed in a GeneAmp PCR System 2400 thermocycler (Applied Biosystems, Waltham, Massachusetts, USA). All PCR reactions had the same parameters, which included polymerase activation at 94 °C/1 min, followed by 40 amplification cycles: denaturation at 94 °C/30 sec; elongation at 68 °C/1 min; final elongation at 68 °C/5 min. Only annealing temperatures were unique: 56 °C for 16s-SC, 51 °C for 16s-BB and 16s-VS461, and 45 °C for 16s-BG, each lasting 30 seconds. PCR products were separated and visualized by agarose gel electrophoresis.

Sequencing of B. burgdorferi s. l. complex amplicons. Sanger sequencing of PCR products was performed using the 16s rRNA gene primer of *B. burgdorferi s. l.* SC (5'-CTTAGCTGCTGCCTCCCGTA-3'). Sequencing was performed at Expogen LLC, Lviv, Ukraine. Sequences were deposited into GenBank (accession numbers: OR532270; OR532271; OR532272; OR532273; OR532274; OR532275; OR532276; OR532277; OR532278; OR532279).

Statistical analysis. Social Science Statistics (www.socscistatistics.com) was used for statistical analysis. Statistical processing of the data was performed using Fisher's exact test for a 2 × 2 data set and the two-sided test. We assessed differences in the regional prevalence of *I. ricinus*, *D. reticulatus*, and *H. marginatum* ticks. We also compared the prevalence of the *B. burgdorferi s. l.* complex both within regions and among ixodid ticks. Comparative analysis of the prevalence of *B. burgdorferi s. s.* and *B. afzelii* genotypes within regions and among *I. ricinus* and *D. reticulatus* ticks was also performed.

Mapping. The maps were created using Microsoft Excel on the basis of Bing, © GeoNames, Microsoft, Navinfo, TomTom, Wikipedia.

Results

Tick collection. A total of 1132 ixodid ticks, from 15 sites across three regions of Ukraine, were examined (Fig. 1). The ticks belonged to the species *I. ricinus* (n = 554), *D. reticulatus* (n = 352) and *H. marginatum* (n = 226). From vegetation, 410 *I. ricinus* ticks and 272 *D. reticulatus* ticks were collected. From host animals of different species, 144 *I. ricinus*, 80 *D. reticulatus*, and 226 *H. marginatum* were collected (Table 1).

Fig. 1. Ixodid tick collection sites, Ukraine (2021): Kyiv region: Kyiv (50°27′00″ N, 30°31′25″ E); Ivankiv (50°56′07″ N, 29°53′46″ E); Fastiv (50°04′29″ N, 29°55′05″ E); Fursy (49°47′59″ N, 30°00′04″ E); Bila Tserkva (49°47′44″ N, 30°07′00″ E); Tetiiv (49°22′15″ N, 29°41′24″ E); Cherkasy region: Korsun-Shevchenkivskyi (49°25′10″ N 31°16′38″ E); Zhabianka (49°12′42″ N 30°45′09″ E); Talne (48°53′10″ N 30°42′09″ E); Bilashky (48°50′27″ N 30°38′40″ E). Mykolaiv region: Blahodatne (47°59′45″ N 31°08′48″ E); Arbuzynka (47°54′24″ N 31°18′47″ E); Bereznehuvate (47°14′39″ N 32°59′25″ E); Mykolaiv city (46°58′31″ N, 31°59′37″ E); Ochakiv (46°37′07″ N, 31°32′21″ E)

Table 1
Numbers and species composition of the studied of ixodid ticks collected in three regions of Ukraine

Region	Questing ixodid ticks		Engorged ixodid ticks		
	I. ricinus	*D. reticulatus*	*I. ricinus*	*D. reticulatus*	*H. marginatum*
Kyiv	339	74	123[a]	44[b]	–
Cherkasy	54	162	16	24	–
Mykolaiv	17	36	5	12	226[c]
Total	410	272	144	80	226

Note: [a] 30 out of 123 *I. ricinus* were collected from wild animals, the remaining 93 were collected from domestic animals, Kyiv region; [b] 16 out of 44 *D. reticulatus* were collected from wild animals, the remaining 28 from domestic animals, Kyiv region; [c] 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 = 288) 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.

Ticks of the species *I. ricinus* showed a statistically significantly higher level of infection with the *B. burgdorferi s. l.* complex at 29.2%

(162/554), compared to *D. reticulatus* ticks at 15.9% (56/352) (P < 0.0001). The total prevalence of the *B. burgdorferi s. l.* complex among questing *I. ricinus* and *D. reticulatus* ticks was 29.8% (203/682), which significantly exceeds the prevalence among engorged *I. ricinus* and *D. reticulatus* ticks – 6.7% (15/224) (P < 0.0001).

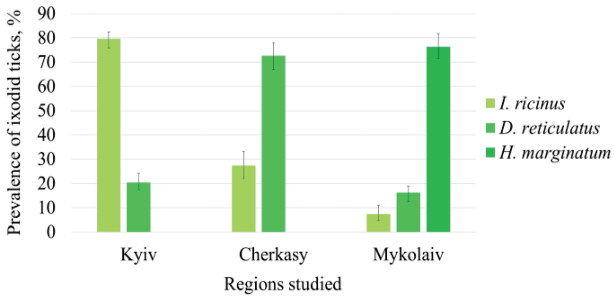

Fig. 2. Species composition of ixodid ticks in three regions of Ukraine

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).

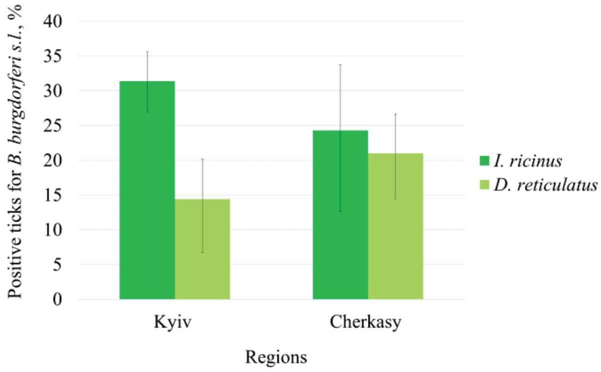

Fig. 3. Prevalence of *B. burgdorferi s. l.* among ixodid ticks in Kyiv and Cherkasy regions, Ukraine

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).

Table 2
Infestation of ixodid ticks with *B. burgdorferi s. l.* complex in three regions of Ukraine

Origin of ticks	*I. ricinus*		*D. reticulatus*	
	PCR-positive ticks / total number of ticks	Prevalence, %	PCR-positive ticks / total number of ticks	Prevalence, %
Kyiv:	–	–	–	–
from vegetation	134/339	39.5	16/74	21.6
from domestic animals	9/93	10.0	1/28	3.6
from wild animals	2/30	0.7	0/16	0.0
Combined	145/462	31.4	17/118	14.4
Cherkasy:	–	–	–	–
from vegetation	16/54	29.6	37/162	22.8
from domestic animals	1/16	6.3	2/24	8.3
Combined	17/70	24.3	39/186	21.0
Mykolaiv:	–	–	–	–
from vegetation	0/17	0.0	0/36	0.0
from domestic animals	0/5	0.0	0/12	0.0
Combined	0/22	0.0	0/48	0.0
Total	162/554	29.2	56/352	16.0

In Kyiv region, out of 580 ticks examined, the prevalence of *B. afzelii* (17.6%) was significantly higher than the prevalence of *B. burgdorferi s. s.* (9.5%, P = 0.0002). However, in Cherkasy region, out of 256 ticks examined, the prevalence of *B. afzelii* (11.0%) did not significantly differ from the prevalence of *B. burgdorferi s. s.* (9.0%, P = 0.5758).

Comparison of the prevalence of *B. afzelii* genotype between Kyiv region (17.6%, 102/508) and Cherkasy region (11.0%, 28/256) did not reveal a statistically significant difference (P = 0.0169). Similarly, there was no statistically significant difference in the combined prevalence of *B. burgdorferi s. s.* between Kyiv – 9.5% (55/580) and Cherkasy – 9.0% (23/256) regions (P = 0.8977, Table 3).

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; Fedoniuk 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).

Table 3
Infestation of *I. ricinus* and *D. reticulatus* ticks with genotypes of the *B. burgdorferi s. l.* in Kyiv and Cherkasy regions

Origin of ticks	*I. ricinus*				*D. reticulatus*			
	B. burgdorferi s.s.		*B. afzelii*		*B. burgdorferi s.s.*		*B. afzelii*	
	PCR-positive / total number [a]	Prevalence, %	PCR-positive / total number	Prevalence, %	PCR-positive / total number	Prevalence, %	PCR-positive / total number	Prevalence, %
Kyiv:								
from vegetation	39/339	11.5	90/339	26.5	7/74	9.5	9/74	12.2
from animals	8/123	6.5	3/123	2.4	1/44	3.6	0/44	0.0
Combined	47/462	10.2	93/462	20.1	8/118	6.8	9/118	7.6
Cherkasy:								
from vegetation	6/54	11.1	9/54	16.7	14/162	8.6	19/162	11.6
from animals	1/16	6.3	0/16	0.0	2/24	8.2	0/24	0.0
Combined	7/70	10.0	9/70	13.0	16/186	8.6	19/186	10.2
Total	54/532	10.2	102/532	19.2	24/304	8.0	28/304	9.2

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

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* 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 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 Southern 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.

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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