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STUDY OF IXODID TICKS IN RECREATIONAL AREAS OF LARGE CITIES IN 2017–2022

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Background. Ixodes ticks are generalist ticks, which can easily adapt to new conditions, and therefore are widespread in urban areas. They are central components for understanding tick-borne disease epidemiology in large cities.

Materials and methods. The main methods of collecting ticks were flagging, which was most often used; ticks were also collected manually by removing them from human and animal bodies. Ticks were examined in the laboratory by dark-field microscopy using optoelectronic systems IMAGLAV-SEO, Leica DM 500 (204), Olimpus, ZEISS Axio Lab A1, and MIKMED-1. PCR studies were performed in real time on a ROTOR Gene 6000 amplifier.

Results. According to the results of field and laboratory studies in the parks of Ternopil during 2018–2019 by the Ternopil Regional Center for Disease Control and

Prevention (TRCDCP) of the Ministry of Health of Ukraine, 524 ticks were collected, 515 of which were examined using dark-field microscopy (DFM) and polymerase chain reaction (PCR). Among the collected specimens, 63 (12.23 %) were the carriers of infectious pathogens, including 24 (4.66 %) carriers of *Borrelia burgdorferi* sensu lato (s.l.) (Johnson et al., 1984). Besides, 7654 ticks removed from humans were examined in the laboratory of the TRCDCP and in the laboratory of the I. Horbachevsky Ternopil National Medical University. Of these, 1280 (16.72 %) specimens were carriers of infectious agents.

The Lviv CDC collected 1314 ticks in recreational areas of Lviv during 2017–2022, of which 139 (10.58 %) were vectors. Of the 1136 ticks collected from humans, 295 (25.96 %) were carriers of *B. burgdorferi* s.l.

Out of 540 ticks collected in parks by the Chernivtsi Regional Center for Tick Control in Chernivtsi, 164 (30.37 %) were infected with *B. burgdorferi* s.l. Out of 454 ticks collected from people in the city, 130 (28.63 %) were infected with *B. burgdorferi* s.l. In the parks of Khmelnytskyi, researchers of the Khmelnytskyi Regional Center for Tick Control collected 5068 tick species, of which 102 (2.01 %) were carriers of *B. burgdorferi* s.l. Spirochetes of *B. burgdorferi* s.l. were detected in 694 (34.87 %) species out of 1990 examined ticks removed from humans.

Conclusions. The dominant tick species in the recreational areas of Lviv, Ternopil, Chernivtsi and Khmelnytsky regions are *Ixodes ricinus* and *Dermacentor reticulatus*. The proportion of ticks collected in city parks that carry infectious agents ranges from 10.58 % to 30.37 %, with the exception of Khmelnytsky, where the tick infestation rate is only 2.01 %. The infection rate of ticks removed from humans with *B. burgdorferi* s.l. and other pathogens ranges from 16.72 % to 34.87 %.

Keywords: Ixodes ticks, dark-field microscopy, PCR studies

INTRODUCTION

Over the last years, Ixodes ticks have begun to increase in number and expand their range from south to north. Currently, the most widespread species, such as *Ixodes ricinus* (Linnaeus, 1758) and *Dermacentor reticulatus* (Fabricius, 1794), are successfully exploiting various biocenoses in northern Europe, in particular in Sweden, Norway and even Iceland (Junttila *et al.*, 1999; Oechslin *et al.*, 2017).

Ukraine, as a country in Eastern Europe, has a temperate continental climate, which is quite suitable for the development of many tick species, including those that until recently were more common to the south.

Several factors contribute to the spread of ticks: climate change, migration of birds, large and small mammals, and human activity. These factors are favorable for the spread of ticks not only in natural biocenoses, but also in agroecosystems and urban biocenoses.

Studies by many Ukrainian and foreign scientists have shown that the process of ticks' expansion in the recreational areas of large and small cities has recently increased significantly, which in turn leads to an increase in the number of urban population infected with tick-borne infections (Akimov & Nebogatkin, 2016; Didyk *et al.*, 2017; Trefanenko *et al.*, 2020). In this regard, there is an urgent need to study ticks in urban recreational areas, their biology and epidemiological significance.

I. A. Akimov and I. V. Nebogatkin (2016) report the results of a study of Ixodes ticks in urban landscapes of Kyiv. Their study presents the distribution of 14 species of ticks within the recreational areas of Kyiv, of which 4 species were discovered for the first

time and 2 species were accidentally introduced. However, of all the identified tick species, only 4–5 species attack humans and are capable of transmitting infectious agents. The authors found that ticks were infected with one type of virus (tick-borne encephalitis virus), 10 types of bacteria: *B. burgdorferi* s.l. (Johnson et al., 1984), *A. phagocytophilum* (Dumler et al., 2001*), Ehrlichia canis*, *Rikettsia helvetica* (Raoult, 1993), *R. monacensis* (Simser et al., 2002), *R. raoulti* (Mediannikov *et al.*, 2008), *Yersinia pseudotuberculosis* (Smith & Thal, 1965), *Francisella tularensis* (McCoy and Chapin, 1912), *Mycoplasma haemocanis* (Messick et al., 2002)*, Ca*. *My. haematoparvum* by two species of protozoa *Babesia* canis (Piana & Galli-Valerio, 1895), *Hepatozoon canis* (Miller, 1908), and larvae of two species of helminths: *Dirophilaria repens* (Railliet & Henry, 1911), *D. immitis* (Leidy, 1856).

The study of ticks in parks of Kyiv illustrated that *I. ricinus* carries such pathogens as *Borrelia burgdorferi sensu lato* (4 % of ticks), *Anaplasma phagocytophilum* (5.2 %), and *Babesia microti* (França, 1912) (1.9 %). The analysis of human cases in Ukraine increased from 1686 in 2014 to 3413 in 2015. At the same time, 67.3 % of patients were from urban areas (Didyk *et al.*, 2017).

The study conducted in Chernivtsi by I. Trefanenko *et al.* (2020) found that in 2018, the percentage of infected ticks from the environment was 34.3 %, and in ticks removed from humans – 17.3 %. In 2019, these figures were slightly higher: the percentage of infected ticks from the environment was 36.2 %, and the percentage of ticks from humans was 31.6 %.

As reported by V. Levytska *et al.* (2021), in 2018–2019, ticks were collected in five studied regions of Ukraine including Ternopil, Chernivtsi and Khmelnitsky, and the prevalence of tick-borne pathogens in *I. ricinus* adults was investigated. It was found that some of these ticks were carriers of *B. burgdorferi* s.l., representatives of the family *Anaplasmataceae, Rickettsia* spp., *Babesia* spp., *Bartonella* spp.

In similar studies, ticks were collected in urban parks and taken from animals concentrated in these parks in Lviv and Ivano-Frankivsk regions. During the research, 215 ticks (22 *I. ricinus* and 193 *D. reticulatus*) were collected and examined in the laboratory. Among *I. ricinus*, 64.0 % of ticks were infected. Among them, 18.2 % were carriers of *Rikettsia* spp. and 54.5 % of *Rikettsia* spp. were carriers of *Candidatus Neoehrlichia mikurensis*, while *A. phagocytophilum and Bartonella* spp. were detected *in* 4.5 % of the ticks examined. Mixed infections were also detected, their share was 22.7 %. Among *D. reticulatus* ticks, 74.6 % were carriers of infections. In particular, *Rikettsia* spp. was found in 30 %, *Candidatus Neoehrlichia mikurensis* – in 54.9 %, *A. phagocytophilum* – in 1.6 %, *Bartonella* spp. in 6.2 %. Mixed infections were present in 18.1 % of ticks of this species (Levytska *et al*., 2019).

Preliminary data on the study of ticks of the species *I. ricinus* removed from humans in Ternopil and Ternopil region are available from L. Fedoniuk and S. Podobivskyi (2019). Attacks on humans by larvae, nymphs, males and females of this tick species were recorded. Female ticks were mostly carriers of *B. burgdorferi*, and nymphs were carriers of A*. phagocytophilum* and *B. miyamotoi*. The larvae could also carry all three infectious agents, and complex transmission, i.e. the presence of two or more pathogens simultaneously, is also possible. Analysis of the epidemiological status of *D. reticulatus* removed from humans and taken from the environment in Ternopil and Ternopil region was made among males, carriage of *B. burgdorferi* s.l. Additionally, studies of tick's number in other cities and regions of Ukraine were carried out (Fedoniuk *et al.*, 2021).

The distribution of ticks of this species was determined in 74 settlements, including in the regional centres of 10 regions of Ukraine: Lviv, Ivano-Frankivsk, Zakarpattia, Ternopil, Volyn, Rivne, Zhytomyr, Chernihiv, Khmelnytskyi, and Vinnytsia. PCR tests revealed *B. burgdorferi* and *A. phagocytophilum* in some tick samples.

Abundant research on ticks in urban and suburban areas was done in large European cities such as Warsaw, Prague, Bratislava, Bonn, and Helsinki. A study of ticks in recreational areas in Helsinki (Finland) revealed that, in 5 districts, the infestation of ticks with *B. burgdorferi* s.l. ranged from 19 to 55 %, with an average of 32 %. *B. afzelii* prevailing in all areas. No isolates of *B. burgdorferi sensu stricto* were detected. Only two ticks were simultaneously infected with two pathogens: *B. afzelii* (Manuela Marin Canica et al., 1994) and *B. garinii* (Baranton et al., 1992) (Junttila *et al.*, 1999).

In 2003, 1394 ticks from 45 locations in urban and suburban areas were examined in Bonn (Germany). The PCR results revealed infection with *B. burgdorferi* s.l. in 250 (17.9 %) ticks. It was found that some infections of *Borrelia genera* (91.4 %) could be attributed to *B. afzelii* (39.5 %), *B. garinii* (27.9 %), *B. burgdorferi* s.l. sensu stricto (15.6 %) and *B. valaisiana* (Wang et al., 1997) (8.6 %) (Maetzel *et al.*, 2005).

A study on the distribution of the *Borrelia* complex *B. burgdorferi* s.l. in *I. ricinus* ticks in natural and urban ecosystems of Ostrava (Czech Republic) reported that the minimum prevalence of *B. burgdorferi* s.l. in ticks for the city park Belskýles was 13.8 % (17.6 % in males, 17.8 % in females and 11.7 % in nymphs). Similarly, for the natural area of Proskovice, the minimum prevalence was 15 % (12.5 % in males, 20 % in females and 14.9 % in nymphs) (Venclíková *et al.*, 2014).

In 2014–2020, similar studies were performed in Prague (Czech Republic). During this period 2819 ticks of *I. ricinus* were studied. 792 species (28.1 %) were positive for *B. burgdorferi* s.l. The infection rate ranged from 24.9 % to 38.5 %. The prevalence of *B. burgdorferi* s.l. was 31 % in males, 33.7 % in females and 25.8 % in nymphs (Richtrová, 2022).

In 2013–2015, ticks were collected in the Warsaw City Park and in two Warsaw suburban forests. According to the results of PCR studies of 493 ticks, their infection with spirochetes of the genus *Borrelia* and in particular *B. miyamoto*i (Fukunaga et al., 1995) species was 17.3 %, including 24.1 % of adults and 2 % of nymphs. In suburban forests, the number of ticks was higher, but the degree of infestation was lower (10.7 % and 8.4 %) (Kovalec *et al.*, 2017).

Later, Polish scientists examined ticks in Warsaw's park areas for other pathogens. It was found that the prevalence of certain *Rickettsiales* in urban areas was twice as high as in natural areas (13.2 % vs. 6.9%, respectively). In general, the prevalence of *Rickettsia* spp., *A. phagocytophilum* and *Candidatus Neoehrlichia mikurensis* in ticks was 6.5 %, 5.3 % and 3.6% in urban areas versus 4.4 %, 1.1 % and 2.1 % in natural areas, respectively (Kowalec *et al.*, 2019).

I. ricinus were also studied in the vicinity of Olsztyn in northeastern Poland. The total infection rate of *I. ricinus* with *Borrelia spirochaetes* was 27.4 %, *B. afzelii* (93.1 %; 27/29), *B. valaisiana* 3.5 % (1/29) and *B. miyamotoi* (3.5 %; 1/29). The infestation rate of adult ticks (42.0 %) was three times higher than that of nymphs (14.3 %) (Kubiak *et al.*, 2019).

I. ricinus, collected during 2015–2016 in 18 urban territories across Switzerland, showed a tick-borne encephalitis virus carriage rate of 0 % for WNV, 18.0 % for *B. burgdorferi* s.l. (8.2 % for *I. ricinus. B. afzelii*, 1.3 % for *B. burgdorferi* s.l. sensu stricto, 2.8 % for *B. garinii*, 0.9 % for *B. valaisiana*, 2.3 % for other *Borrelia* species), 2.5 %

for *B. miyamotoi*, 13.5 % for *Rickettsia* spp. (13.2 % for *R. helvetica*, 0.3 % for *R. monacensis*), 1.4 % for *A. phagocytophilum*, 6.2 % for *Candidanus Neoehrlichia mikurensis* and 0.8 % for *Babesia venatorum* (Oechslin *et al.*, 2017).

In the park areas of Bratislava, 932 *I. ricinus* ticks were examined for pathogens: *B. miyamotoi*, *B. burgdorferi* s.l., *A. phagocytophilum* and *R. helvetica*. The overall prevalence of *B. miyamotoi, B. burgdorferi* s.l., *A. phagocytophilum* and *R. helvetica* was 0.75 %, 13.2 %, 5.6 % and 8.9 %, respectively. *B. afzelii* was the most common species, followed by *B. garinii* and *B. valaisiana* (Vaculová *et al.*, 2019).

The analysis of the results of research by domestic and foreign scientists showed the need to study the situation with ticks and tick-borne pathogens in urban and suburban areas of some cities in the west of Ukraine.

The aim of this paper was to analyze the results of the study of ticks in recreational areas of large cities in the west of Ukraine: Ternopil, Lviv, Chernivtsi, and Khmelnytsky.

MATERIALS AND METHODS

When working in the field, the flagging method of collecting ticks was most often used. Indicators of abundance were expressed in the number of specimens per 1 flag/km (abundance index). Ticks were identified to species according to the identification tables presented in the monograph by E. M. Yemchuk (1960) and I. A. Akimov (2016). Ticks were also collected manually by removing them from clothes, from the bodies of people and animals.

A total of 7698 specimens of two species of Ixodes ticks, *I. ricinus* (6081 specimens) and *D. reticulatus* (1617 specimens), were collected and examined in the park areas of Ternopil, Lviv, Khmelnytsky and Chernivtsi in 2017–2022. Also, during the same period, 11296 specimens of ticks of both species were collected from humans and examined (11171 specimens and 125 specimens, respectively).

Ticks removed from people who had been attacked by ticks were examined by the Laboratory for Ticks and Tick-Borne Infections of the I. Horbachevsky Ternopil National Medical University (TNMU) and the laboratories of especially dangerous infections of the Regional Centers for Disease Control and Prevention of the Ministry of Health of Ukraine in Lviv, Ternopil, Khmelnytsky and Chernivtsi.

In the Laboratory of the Center for Tick Control of the Ministry of Health of Ukraine, ticks were identified and examined for infectious agents using optoelectronic systems IMAGLAV-SEO, Leica DM 500 (204), Olimpus, ZEISS Axio Lab A1, MIKMED-1 using the dark field microscopy (DFM) method (Order of the Ministry of Health of Ukraine No. 218, 2005). In the Chernivtsi Regional Center for Tick-Borne Disease Control and Prevention, the detection of *Borrelia* in ticks and the assessment of the degree of individual infection of ticks was carried out by the method of rapid indication of *Borrelia* (dark-field microscopy) (Biletska & Semenyshyn, 2002).

The material for the study were Ixodes ticks. The study was carried out on the day of tick delivery using the ICC method, which is a reliable way to indicate *Borrelia* in tick vectors. Samples for examination were made from the arthropods using a degreased slide no more than 1.2 mm thick and coverslips 18×18 mm in size, and 250 to 500 fields of view of each specimen were examined. Different optical systems were used: in MIKMED-1 microscopes with AU-12 binocular objectives and ZEISS Axio Lab A1 microscopes – 600× magnification (40×1.5×10), in Leica DM500 and Olimpus microscopes – 400× magnification (10× eyepiece, 40× objective) with a dark-field condenser.

The search for *Borrelia* was carried out only in the thinnest marginal part of the drop, in a strip not exceeding the diameter of one field of view. In the presence of spirochetes (*Borrelia*), their total number was counted. The concentration of *Borrelia* in the preparation was expressed as the average number of microbial bodies per 100 fields of view. The degree of infection of ticks with *Borrelia* was calculated using a 3-point system:

- low – from single *Borrelia* to 10 per 100 fields;
- medium – from 10 and more per 100 fields of view;
- high – in the presence of *Borrelia* accumulations in the form of „tangles", „braids" in combination with single specimens in almost every field of view.

Determination of DNA/RNA pathogens by polymerase chain reaction (PCR) [MR, Kyiv, 2007, DSP 9.9.5-153-2008] was performed both at TNMU and the UCLC. Several commercial test systems were used: „DNA *A. phagocytophilum* / DNA *E. muris* / *E. chaffeensis* DNA"; „*B. burgdorferi* s.l. DNA"; „*B. myamotoi*DNA"; „*Babesia species* DNA"; „*Ticks borne of encephalitis viruses*RNA" (manufactured by MolGen (Italy), allowing real-time detection on the ROTOR Gene 6000 amplifier). Statistical data processing was performed using SPSS (IBM, USA). In cases where the Shapiro–Wilk test indicated a normal distribution of data, the reliability of the indicators was assessed by Student's *t*-test. In case of non-normal distribution, the Mann–Whitney test was used. Differences with ** – $p \leq 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

The study was carried out in two ways: the study of ticks collected directly in parks, squares and small urban forest plantations using the flagging method and the study of ticks removed from people who were attacked by ticks in recreational areas of cities.

In Ternopil, tick collections were conducted in two seasons – spring and fall in 2018–2019 in five parks: Natsional'ne Vidrodzhennya, Shevchenko, Topilche, Kutkivtsi, and Zdorovya. A total of 524 specimens of *I. ricinus* ticks were collected, of which 515 specimens were examined using ICC and PCR.

We found 63 (12.23 %) specimens that were carriers of infectious pathogens, including 24 (4.66 %) ticks that were carriers of *B. burgdorferi,* 15 (2.91 %) – *A. phagocytophilum,* 7 (1.36 %) – *B. miyamotoi* and 17 (3.30 %) – *Babesia* spp. (**Table 1**).

Most ticks were collected in Kutkivtsi Park – 229 specimens with an abundance index of 21.26. Out of 226 ticks examined by PCR and ICC, 7.52 % of ticks carrying infectious agents were detected. In particular, the infection rate of *B. burgdorferi* was 2.65 %, *B. miyamotoi* – 0.88 %, *A. phagocytophilum* – 2.21 %, *Babesia* spp. – 1.77 %.

In Natsional'ne Vidrodzhennya park, 176 specimens were collected with an abundance index of 6.96, of which 170 specimens were examined; 18.24 % of them were found to be infected with pathogens, including *B. burgdorferi* – 8.24 %, *B. miyamotoi* – 1.76 %, *A. phagocytophilum* – 4.12 % and *Babesia* spp.

In Zdorovya park, 63 ticks were collected and examined with an abundance index of 7.4, of which 9.52 % were carriers of infections. *B. burgdorferi* was detected in 4.76 % of ticks, *A. phagocytophilum* and *Babesia* spp. in 1.59 % of ticks. In Topilche park, 52 ticks were collected with an abundance index of 2.73. Infections were found in 19.23 % of ticks: *B. burgdorferi* – 3.85 %, *B. miyamotoi* – 3.85 %, *A. phagocytophilum* – 3.85 %, *Babesia* spp. – 7.69 % (**Table 2**).

Only a few tick specimens were found in Shevchenko Park, as the park is based on old trees and with barely any shrubs.

Table 1. **Results of tick surveys in recreational areas of Ternopil, Lviv, Chernivtsi and Khmelnytsky in 2017–2022**

Date of collection (year)	Place of collection (city)	Tick species	Total number of examined ticks	Total number of infected ticks	Research methods		Pathogens identified			
					PCR	Dark-field microscopy	Bb	Bm	A	Babesia
2017	Lviv	*D. reticulatus*	276	31 (11.23 %)	–	276	31	–	–	–
	Chernivtsi	*I. ricinus*	132	13 (9.85±0.06 %)	–	132	13	–	–	–
	Khmelnytsky	*I. ricinus*	377	15 (3.98±0.03%)	87	290	15	–	–	–
2018	Ternopil 2	*I. ricinus*	385	34 (8.83 %)	203	182	16	4	8	6
	Lviv	*D. reticulatus*	200	16 (8.0 %)	–	200	16	–	–	–
	Chernivtsi	*I. ricinus*	104	37 (35.58±0.03 %)	–	104	37	–	–	–
	Khmelnytsky	*I. ricinus*	344	18 (5.23 %)	8	10	18	–	–	–
2019	Ternopil 2	*I. ricinus*	130	27 (20.77±0.04%)	95	35	9	4	7	7
	Lviv	*D. reticulatus*	360	23 (6.39±0.02 %)	–	30	23	–	–	–
	Chernivtsi	*I. ricinus*	87	42 (48.28±0.03 %)	–	87	42	–	–	–
	Khmelnytsky	*I. ricinus*	2548	46 (1.81±0.05 %)	31	15	46	–	–	–
2020	Lviv	*D. reticulatus*	57	4 (7.02±0.03 %)	–	57	4	–	–	–
	Chernivtsi	*I. ricinus*	27	12 (44.44 %)	–	27	12	–	–	–
	Khmelnytsky	*I. ricinus*	–	–	–	–	–	–	–	–
2021	Lviv	*D. reticulatus*	380	35 (9.21 %)	–	380	35	–	–	–
	Chernivtsi	*I. ricinus*	107	35 (32.71 %)	–	107	35	–	–	–
	Khmelnytsky	*I.ricinus*	907	10 (1.1 %)	873	34	10	–	–	–
2022	Ternopil 2	*D. reticulatus*	344	22 (6.40±0.01 %)	–	344	22	–	–	–
	Lviv	*I. ricinus*	83	25 (30.12 %)	–	83	25	–	–	–
	Chernivtsi	*I. ricinus*	892	13 (1.46±0.05 %)	737	155	13	–	–	–
2017–2022	Total	*D. reticulatus*	1617	131 (8.1 %)	–	1287	131	–	–	–
		I. ricinus	6081	327 (5.38±0.03%	2043	1210	291	8	15	13

Note 1: Bb – *Borrelia burgdorferi* s.l.; Bm – *Borrelia miyamotoi*; A – *Anaplasma phagocytophilum*

Note 2: Ternopil 2 – results of the research conducted by the Ternopil Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine

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Table 2. **The results of studies on the detection of spirochetes of *B. burgdorferi* s.l. in ticks removed from humans in the laboratories of the Ternopil Regional Center for TNMU in 2017–2019**

Years	Laboratory of Ternopil RDCKP	Laboratory of Ternopil TNMY	Difference
2017	15.15±0.06 %	12.20 %	2.95 %
2018	15.49±0.02 %	14.79±0.05 %	0.66 %
2019	13.78±0.05 %	14.10±0.01 %	0.32 %

In the recreational areas of Lviv (Vysokyi Zamok, Pohulyanka, Levandivskyi, Piskovi Ozera, Kortumova Hora, Stryiskyi, Ivan Franko, Sykhivskyi, etc. parks), 1617 specimens of *D. reticulatus* ticks were collected by flagging and tested by the ICC method during 2017-2022, of which 131 specimens, about 8.1 %, were infected with borreliosis pathogens. The index of abundance ranged from 0.91 to 1.12 and averaged 0.94 specimens per flag/km (**Table 1**).

In the context of the recent years, the results were as follows: in 2017, 276 ticks were collected, of which 31 were carriers of *B. burgdorferi* s.l., which corresponded to 11.23 %. In 2018, out of 200 ticks, 16 (8.0 %) were infected with *B. burgdorferi* s.l. In 2019, these figures were as follows: out of 360 ticks examined, 23 (6.39 %) were carriers of *B. burgdorferi* s.l. In 2020, only 4 out of 57 ticks were infected (7.01 %). In 2021, 35 (9.21 %) of 380 ticks examined were infected, and in 2022, 22 (6.4 %) of 344 ticks were carriers of *B. burgdorferi* s.l. (**Table 2**). It is worth noting that in the recreational areas of Lviv, the lion's share of ticks was made up of *D. reticulatus* species, unlike in other cities, where *I. ricinus* species dominated. At the same time, the highest rates of *Borrelia* carriage from year to year were observed in Sykhivskyi (11.2 %), Vysokyi Zamok (15.0 %), Levandivskyi (9.09 %), and Piskovi Ozera (10.0 %) parks.

When monitoring the spread of ticks in recreational areas of Chernivtsi, the territories of 5 parks, 2 forest belts and 2 forests, which are nominally part of the city territory, were examined. A total of 540 species of *I. ricinus* ticks were collected by flagging and examined by the ICC. It was found that 164 specimens (30.37 %) were infected with spirochetes. In terms of years, it looked as follows: in 2017, 13 specimens out of 132 were found to be infected, which was 9.85 % of the studied ticks; in 2017 – 37/104 (35.58 %); in 2019 – 42/87 (48.27 %); in 2020 – 12/27 (44.44 %); in 2021 – 35/107 (32.7 %) and in 2022 – 25/83 (30.12 %) (**Table 1**).

The largest forest park area in Chernivtsi is the territory of the Salhirskyi district of the city, which is more like a forest than a park. Here, 421 ticks were collected and examined, of which 124 (29.45 %) were carriers of *Borrelia*. In other forest park areas, 43 specimens were collected, of which 11 (26.19 %) were carriers of *B. burgdorferi* s.l. In general, 464 specimens of *I. ricinus* ticks were collected in biocenoses more similar to forests, of which 135 specimens (29.09 %) were found to carry *B. burgdorferi* s.l.

In typical park areas of Chernivtsi, 76 ticks were collected, of which 29 (38.16 %) were infected with *B. burgdorferi* s.l. It can be seen that the number of *I. ricinus* ticks in Chernivtsi parks is much lower, but their infection rate with borreliosis pathogens is much higher than in forest park areas. The vast majority of collected ticks were concentrated in more natural biocenoses (**Table 1**).

In park areas of Khmelnytsky, 4998 specimens of *I. ricinus* ticks were collected in 2017–2022. According to the results of their examination, the ICC found that 111 spe-

cies, or 2.22 %, were carriers of *B. burgdorferi* s.l. In the context of the recent years, the distribution of ticks and the degree of their infection was as follows: in 2017, 24 species out of 377 ticks examined were infected with *B*. *burgdorferi* s.l., which corresponds to 6.37 %, in 2018 – 18/34 (5.23 %), in 2019 – 46/2478 (1.08 %), in 2021 – 10/907 (1.1 %) and in 2022 – 13/892 (1.46 %) (**Table 1**).

It is noteworthy that the proportion of *I. ricinus* ticks infected with *Borrelia* in parks of Khmelnytsky was significantly lower than in other cities (Chernivtsi, Ternopil, Lviv).

In order to assess the risk of Lyme borreliosis and other infections in people, who have been attacked by ticks, studies were conducted to identify infectious agents in them.

In Ternopil, a total of 7277 ticks removed from people were examined during 2017–2022, of which 1202 (16.52 %) were carriers of infectious agents. In particular, *B. burgdorferi* s.l. was detected in 1064 (11.47 %) ticks, *A. phagocytophilum* – in 93 (1.0 %), *B. miyamotoi* – in 5 (0.05 %), *B. burgdorferi* together with *A. phagocytophilum* – in 34 (0.38 %), *B. burgdorferi* s.l. together with *B. miyamotoi* – in 4 (0.04 %) and *B. miyamoto*i together with *A. phagocytophilum* – in 2 (0.02 %) (**Table 3**).

In the laboratory of the Ternopil Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine, 6396 specimens *of I. ricinus* ticks removed from humans were examined by the dark-field microscopy method. The total number of infected ticks was 945 specimens (14.77 %) and all of them were carriers of *B. burgdorferi* s.l.

In the laboratory for the study of ticks and tick-borne infections of TNMU, 881 specimens of *I. ricinus* were examined by PCR, of which 256 specimens (29.0±0.05 %) were infected with infectious agents.

To verify the reliability of laboratory tests conducted in parallel in both laboratories, the results of detection of *B. burgdorferi* s.l. in ticks removed from humans during 2017–2019 were compared (**Table 2**).

According to the results obtained, the difference between the results of the two laboratories is within the permissible range of $p \leq 0.01$, so it can be concluded that the parallel laboratory studies reflect the real state of infection of ticks with spirochetes *B. burgdorferi* s.l. within the city of Ternopil.

The TNMU laboratory analyzed the infection with some pathogens at two stages of tick development – adults and nymphs, which most often attack humans. The results of such comparisons of infestation of nymphs and adults of *I. ricinus* ticks were as follows: on average, 22.53 % of females and 12.38% of nymphs were carriers of *B. burgdorferi* s.l. *A. phagocytophilum* was detected in 15.64 % of females and 4.25 % of nymphs. Co-infection with *B. burgdorferi* s.l. + *A. phagocytophilum* was observed in 4.5 % of females and 5.1 % of nymphs. A very small number of ticks had a combination of two or three other pathogens, and the frequency of such combinations was very low (**Table 3**).

The laboratory of the Lviv Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine examined 1448 specimens of *I. ricinus* and 125 specimens of *D. reticulatus* ticks removed from humans. The ICC method revealed that 366 specimens (25.24 %) of *I. ricinus* ticks and 19 specimens (15.45 %) of *D. reticulatus* ticks were carriers of *B. burgdorferi* s.l. In terms of years, the rates of infection of ticks of both species with borreliosis pathogens were as follows: in 2017 – 99 specimens or 24.57 % for *I. ricinus*, in 2018 – 99 species or 24.87 % for *I. ricinus* and 2 species or 9.09 % for *D*. *reticulatus,* in 2019 – 90 (33.33 %) species for *I. ricinus* and 10 (26.32 %) for *D. reticulatus*, in 2020 – 18 (17.48 %) for *I. ricinus* and 4 (11.43 %) for *D*. *reticulatus*, in 2021 – 27 (17.31 %) for *I. ricinus* and 2 (9.09 %) for *D. reticulatus*, and in 2022 –

Table 3. **Results of tests on ticks collected from people in the cities of Ternopil, Lviv, Chernivtsi and Khmelnytskyi in 2017–2022**

Date of collection (year)	Place of collection (city)	Tick species	Total ticks examined	Total infected ticks	Examination methods		Pathogens detected					
					PCR	Dark-field microscopy	Bb	Bm	A	Bb + A	Bb + Bм	Bm + A
2017	Ternopil 1	*I. ricinus*	295	74 (25.08±0.04 %)	295	–	36	3	18	14	1	2
	Ternopil 2	*I. ricinus*	1558	236 (15.15±0.03 %)	1	235	236	–	–	–	–	–
	Lviv	*I. ricinus*	405	99 (24.44±0.04 %)	–	405	99	–	–	–	–	–
		D. reticulatus	2	–	–	2	–	–	–	–	–	–
	Chernivtsi	*I. ricinus*	85	23 (27.06±0.02 %)	–	85	23	–	–	–	–	–
	Khmelnytsky	*I. ricinus*	354	112(31.64±0.02 %)	3	351	112	–	–	–	–	–
2018	Ternopil 1	*I. ricinus*	257	92 (35.8 ± 0.01%)	257	–	38	–	40	14	–	–
	Ternopil 2	*I. ricinus*	2486	385 (15.49±0.04%)	–	385	385	–	–	–	–	–
	Lviv	*I. ricinus*	398	99 (24.87±0.4%)	–	398	99	–	–	–	–	–
		D. reticulatus	22	2 (9.1±0.01 %)	–	22	2	–	–	–	–	–
	Chernivtsi	*I. ricinus*	137	32 (23.36±0.05 %)	–	137	32	–	–	–	–	–
	Khmelnytsky	–	568	190 (33.45 %)	2	566	190	–	–	–	–	–
2019	Ternopil 1	*I. ricinus*	227	70 (30.84±0.03%)	227	–	32	1	31	3	2	–
	Ternopil 2	*I. ricinus*	2352	324 (13.78±0.05 %)	54	310	324	–	–	–	–	–
	Lviv	*I. ricinus*	270	90 (33.33 %)	–	270	90	–	–	–	–	–
		D. reticulatus	38	10 (26.32±0.05%)	–	38	10	–	–	–	–	–
	Chernivtsi	*I. ricinus*	56	19 (33.93±0.02 %)	–	56	19	–	–	–	–	–
	Khmelnytsky	*I. ricinus*	472	186 (39.41±0.04%)	–	472	186	–	–	–	–	–

End of the Table 3

2020	Ternopil 1	*I. ricinus*	50	11 (22 %)	50	–	6	1	3	1	–	-
	Lviv	*I. ricinus*	103	18 (17.48±0.03%)	–	103	18	–	–	–	–	–
		D. reticulatus	35	4 (11.43±0.02%)	–	35	4	–	–	–	–	-
	Chernivtsi	*I. ricinus*	69	19 (27.54±0.07%)	–	69	19	–	–	–	–	–
	Khmelnytsky	*I. ricinus*	–	–	–	–	–	–	–	–	–	–
2021	Ternopil 1	*I. ricinus*	41	5 (12.20±0.01%)	41	–	4	–	1	–	–	–
	Lviv Chernivtsi	*I. ricinus*	156	27 (17.31±0.03%)	–	156	27	–	–	–	–	–
		D. reticulatus	22	2 (9.09±0.01%)	–	22	2	–	–	–	–	–
	Khmelnytsky	*I. ricinus*	41	15 (36.59±0.05%)	–	41	15	–	–	–	–	–
	Ternopil 1	*I. ricinus*	276	97 (35.14 %)	–	276	97	–	–	–	–	–
2022	Lviv	*I. ricinus*	11	5 (45.46±0.05%)	11	–	3	–	2	–	–	–
	Chernivtsi	*I. ricinus*	118	33 (27.97±0.03%)	–	118	33	–	–	–	–	–
		D. reticulatus	6	1 (16.67±0.04%)	–	6	1	–	–	–	–	–
	Khmelnytsky	*I. ricinus*	66	22 (33.33 %)	–	66	22	–	–	–	–	–
	Total	*I. ricinus*	320	109 (34.1±0.04 %)	–	320	109	–	–	–	–	–
2017–2022	Ternopil 1	*D. reticulatus*	125	19 (15.2 %)	–	125	19	–	–	–	–	–
		I. ricinus	11171	2391 (21.41 %)	940	4819	2254	5	95	32	3	2

Note 1: Bb – *Borrelia burgdorferi* s.l.; Bm – *Borrelia miyamotoi*; A – *Anaplasma phagocytophilum*

Note 2: Ternopil 1 – the results of the research conducted by the laboratory for the study of ticks and tick-borne infectionsy TNMU; Ternopil 2 – the results of the research conducted by the Ternopil Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine

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33 (27.97 %) and 1 (16.67 %), respectively (**Table 3**). It is obvious that the infection rate of *D. reticulatus* ticks was significantly lower than that of *I. ricinus* ticks. At the same time, *D. reticulatus* ticks removed from humans were found in the studies only in Lviv.

In Chernivtsi, 130 specimens of *I. ricinus* ticks infected with *B. burgdorferi* s.l. spirochetes out of the 454 examined ones were detected during the study of ticks removed from humans by the ICC method, which corresponded to 28.63 %. The analysis showed that in 2017, 23 ticks out of 85 examined were infected, which amounted to 27.06 %. In 2018, 32 out of 137 (23.36 %), in 2019 – 19 out of 56 (33.93 %), in 2020 - 19 out of 69 (27.54 %), in 2021 – 15 out of 41 (36.59 %), and in 2022 – 22 out of 66 (33.33 %) ticks examined (**Table 3**).

Ticks removed from humans were infected with *B. burgdorferi* s.l. at almost the same level (in %) as ticks collected in recreational areas of Chernivtsi by flagging.

The laboratory of the Khmelnytsky Regional Center for Disease Control and Prevention of the Ministry of Health of Ukraine examined 2370 specimens of *I. ricinus* ticks removed from humans, and found that 904 specimens (38.14 %) were infected with *B. burgdorferi* s.l. In the context of separate years, the results were as follows: in 2017, 112 infected ticks out of 354 were detected, which corresponded to 31.63 %, in 2018 – 190 out of 568 (33.45 %), in 2019 – 186 out of 472 (39.41 %), in 2020 – no tick tests were conducted, in 2021 – 97 out of 276 (35.14 %) and in 2022 – 109 out of 320 (34.87 %) of the examined individuals (**Table 3**). The percentage of ticks infected with borreliosis pathogens removed from people was an order of magnitude higher than those collected by flagging in parks in Khmelnitsky.

CONCLUSIONS

In 2017–2022, *Ixodes ricinus* and *Dermacentor reticulatus* were the main tick species in the recreational areas of Lviv, Ternopil, Khmelnytsky, and Chernivtsi. These results correspond to information on the distribution of certain tick species in major European cities: Prague, Warsaw, Bratislava, Bonn, and Helsinki, as well as scientific information from previous studies by Ukrainian authors in other regions.

It was found that both species of Ixodes ticks inhabiting recreational areas of large cities in the west of Ukraine are carriers of infectious diseases, primarily borreliosis (in the range of 1.1–44.44 %), as well as anaplasmosis (about 4 %) and babesiosis (about 3.5 %). Most often, infection with *B. burgdorferi* s.l. vectors was detected. In Ternopil, these parameters reached 8-10 % of the total number of ticks examined, in Lviv – an average of about 10.58 %, in Khmelnytsky – 2.01 %, and in Chernivtsi – 30.37 %.

In all cities, the level of infection of ticks removed from people with infectious agents was significantly higher (for *D. reticulatus*, the average was 15.2 %, and for *I. ricinus*, 21.41 %) than that of ticks collected in recreational areas (for *D. reticulatus*, 8.1%, and for *I. ricinus*, 5.38 %). In Ternopil, it was 16.72 %, including carriers of *B. burgdorferi* s.l. – 39.24 %, *A. phagocytophilum* – 32.99 %. In Lviv, the level of infection with *B. burgdorferi* s.l. was 25.96 % on average, in Chernivtsi – 28.63 %, in Khmelnytsky – 34.87 %. These data confirm the conclusions of foreign authors about a greater aggressiveness of infected ticks towards humans.

The results of the research are important in warning the public about the possibility of tick infestation within recreational areas and the danger of being infected with many infectious agents transmitted by these ticks.

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ADHERENCE TO ETHICAL STANDARDS

Conflict of interest. The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ticks were collected in the wild using the flagging method, and ticks were removed from people in hospital emergency rooms. The collected ticks were immediately euthanized using chloroform or diethyl ether. The biological ethics of animal treatment was not violated.

Human rights were not violated, as no human experiments were conducted.

AUTHORS' CONTRIBUTIONS

Conceptualisation, [S.P.; V.P.; O.S.; L.F.]; methodology, [O.C.; L.T.; N.G.; O.S.]; formal analysis, [L.G.; L.S.; V.O.]; research, [S.P.; L.T.; V.P.; O.S.]; data curation, [S.P.; V.P.; N.G.; L.T.]; writing – reviewing and editing, [S.P.; L.F.; O.S.]; supervision, [L.F.; O.C.].

All authors have read and agreed with the published version of the manuscript.

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ДОСЛІДЖЕННЯ ІКСОДОВИХ КЛІЩІВ РЕКРЕАЦІЙНИХ ЗОН ВЕЛИКИХ МІСТ У 2017–2022 РОКАХ

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Вступ. Іксодові кліщі дуже пластичні, легко пристосовуються до нових умов, а тому широко розповсюджені в міських поселеннях і становлять загрозу зараження населення цими збудниками безпосередньо в населених пунктах, зокрема, великих містах.

Матеріали та методи. Під час роботи в польових умовах найчастіше використовували метод збору кліщів на "прапорець". Також їх збирали вручну, знімаючи з одягу, з тіл людей і тварин.

У лабораторіях центрів кліщів досліджували методом темнопольної мікроскопії, використовуючи оптико-електронні системи IMAGLAV-SEO, Leica DM 500 (204), Olimpus, ZEISS Axio Lab A1 та МІКМЕД-1. ПЛР-дослідження проводили в режимі реального часу на ампліфікаторі ROTOR Gene 6000.

Результати. За результатами польових і лабораторних досліджень у парках Тернополя протягом 2018–2019 рр. Тернопільським обласним центром контролю та профілактики хвороб (ОЦКПХ) МОЗ України було зібрано 524 кліщі, з яких 515 екземплярів дослідили за допомогою мікроскопії в темному полі (МТП) і полімерно-ланцюгової реакції (ПЛР). Виявлено 63 (12,23 %) екземпляри, які були носіями інфекційних збудників, зокрема, 24 (4,66 %) кліщі були носіями *Borrelia burgdorferi*. У лабораторії Тернопільського ОЦКПХ і лабораторії з дослідження кліщів та кліщових Тернопільського національного медичного університету ім. І. Я. Горбачевського

було досліджено 7654 кліщів, знятих з людей. З них 1280 (16,72 %) екземплярів були переносниками збудників інфекцій.

У рекреаційних зонах м. Львова у 2017–2022 рр. Львівським ОЦКПХ було зібрано 1314 кліщів, з яких 139 (10,58 %) були переносниками. З 1136 кліщів, знятих з людей, 295 (25,96 %) були носіями *B. burgdorferi* s.l. 164 (30,37 %) з 540 кліщів, зібраних у парках Чернівецьким ОЦКПХ у м. Чернівці, були інфіковані *B. burgdorferi* s.l. З 454 кліщів, знятих з людей в межах цього міста, 130 (28,63 %) були носіями *B. burgdorferi* s.l. У парках м. Хмельницького науковими працівниками Хмельницького ОЦКПХ було зібрано 5068 екземплярів кліщів, з яких 102 (2,01 %) були носіями *B. burgdorferi* s.l. Спірохети *B. burgdorferi* s.l. були виявлені у 694 (34,87 %) екземплярах з 1990 досліджених кліщів, знятих з людей.

Висновки. Основними кліщами, що населяють рекреаційні зони Львівської, Тернопільської, Чернівецької і Хмельницької областей, є *Ixodes ricinus* та *Dermacentor reticulatus*. Частка кліщів, зібраних у парках міст, які є переносниками збудників інфекцій, коливається від 10,58 % до 30,37 %, за винятком м. Хмельницький, де показник інвазійності кліщів становить лише 2,01 %. Під час аналізу кліщів, знятих з людей, їхня інфікованість *B. burgdorferi* s.l. та деякими іншими збудниками коливається від 16,72 % до 34,87 %.

Ключові слова: приміська зона, іксодові кліщі, *B. burgdorferi* s.l., темнопольна мікроскопія, ПЛР-дослідження

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