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Infestation of *Ixodes ricinus* with *Babesia* spp. in Natural and Anthropogenic Habitats of Kharkiv Region and Its Relationship with the Detection of Canine Babesiosis

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ABSTRACT

Among parasitic diseases in dogs, babesiosis plays a significant role as a natural-focal, tick-borne hemoparasitic disease caused by protozoa of the genus *Babesia*, which invade erythrocytes. The present study aimed to determine the prevalence of *Ixodes ricinus* ticks infected with *Babesia* spp. and to assess the epizootiological characteristics of canine babesiosis distribution in various districts of Kharkiv region and the city of Kharkiv during 2012–2023, taking into account seasonal dynamics, age-related susceptibility of dogs, and conditions of the urban environment. A total of 2,690 *I. ricinus* ticks were collected from natural habitats: 1,955 from the Kharkiv Region and 735 from the city, including 294 obtained from dogs. Tick sampling was conducted in parks and residential areas using the flagging method (2 km routes, May 10–20). Ticks were examined morphologically to determine developmental stage and species. In total, 1,486 dog blood samples were analyzed (578 from the region and 908 from the city). *Babesia* parasites were identified via microscopy of methanol-fixed, Giemsa-stained thin blood smears from the ear vein. Samples were positive if *Babesia* trophozoites or paired pyriform merozoites were observed in erythrocytes. *I. ricinus* ticks infected with *Babesia* were found in all studied areas. From 2012 to 2023, average infection rates were 27.8% for nymphs and 35.8% for imago. The infection rate among dogs following tick bites was 36.9%. In the city parks, 48.9% of ticks were infected, compared to 39.7% in residential zones. Among ticks removed from dogs, 52.4% carried *Babesia*, and 46.1% of bitten dogs had babesiosis. The age group most affected in the region was dogs aged 5-6 years (37.7%), followed by 2-4 years (28.8%) and 7-9 years (25.3%). In Kharkiv city, the highest incidence was also in 5-6-year-old dogs (50.7%). Puppies (6 months to 1 year) and dogs over 10 years indicated the lowest rates of incidence (below 5%). Seasonal peaks occurred in April–May (18.3-22.7%) and September (16.1%), indicating clear patterns of disease activity. Thus, babesiosis remains a relevant parasitic disease in dogs in the region, requiring continuous epizootiological monitoring and effective prevention.

Keywords: Babesiosis, Dog, Imago, Nymph, Tick

INTRODUCTION

Despite overall improvements in socio-economic conditions and increased public awareness of preventive measures, parasitic diseases remain a pressing medical and social issue, accounting for a significant proportion of the overall morbidity structure in many regions of the world (Gómez-Muñoz, 2021; Paliy et al., 2022). Since more than 50% of human infectious diseases are of zoonotic origin, it is necessary to further investigate the relationship between wildlife, domestic animals, and humans, as well as focus on targeted studies of the transmission routes of zoonotic pathogens (Diakou et al., 2023). Environmental contamination with parasitic agents increases the risks of disease spread to previously safe areas (Paliy et al., 2019). Zoonotic transmissible diseases are the subject of the application of the One Health concept principles (Giannelli et al., 2024). Humans and companion animals are accidentally caught in the epizootic and epidemic cycle of transmissible diseases (Gonzalez et al., 2014). The pathogens are transmitted through the bites of Ixodes ticks, which are part of natural foci. Ticks can be infected with protozoa that cause babesiosis (Onyiche et al., 2021). Ticks have been reported to transmit anaplasmosis, typhus, and encephalitis (Ghai et al., 2022).

Babesiosis is a vector-borne disease caused by protozoa of the genus *Babesia* and transmitted by the same species of *Ixodes* (black-legged) ticks that transmit Lyme disease (Sosa et al., 2021). Babesiosis is usually a disease of animals, but in recent years, the disease has also been reported in humans. In 2019, 2,418 cases of babesiosis were reported in the United States in states where black-legged ticks are common (Gray and Herwaldt, 2019; Yang et al., 2021). In the United States, *Babesia microti* predominantly infects populations residing in coastal and island regions of Massachusetts, Rhode

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Island, Connecticut, and New York, including eastern Long Island and Shelter Island), as well as in parts of New Jersey, Wisconsin, and Minnesota (Eisen et al., 2017; Krause, 2019; Wolf et al., 2020).

Another species of *Babesia*, *B. divergens*, has been identified as the causative agent of human infections in Missouri, Washington, and California, as well as in various other regions globally (Herwaldt et al., 2004; Gray, 2006). In Europe, human babesiosis is considered an emerging infectious disease (Bonsergent et al., 2021; Hildebrandt et al., 2021).

Certain diseases caused by pathogens of the genera Borrelia, Anaplasma, Rickettsia, and *Babesia* are spreading due to the expanding range of tick vectors (Podobivskiy et al., 2024). The occurrence and incidence of babesiosis has been observed in 20 European countries located in southeastern Europe (Bosnia and Herzegovina, Croatia and Serbia), central Europe (Austria, Czech Republic, Germany, Hungary, Luxembourg, Poland, Slovakia, Slovenia and Switzerland), and northern and northeastern Europe (Lithuania, Latvia, Estonia, Iceland, Denmark, Finland, Sweden and Norway; Bajer et al., 2022).

Babesia enter the bloodstream of animals with the tick's saliva during a bite and can infect a wide range of vertebrate hosts capable of maintaining the transmission cycle (Homer et al., 2000). The primary risk factors for canine infection with *Babesia* spp. include residence in rural areas, stays in animal shelters, presence in endemic regions, seasonal patterns associated with increased activity of infected ticks, and the lack of acaricidal treatment (Su et al., 2023). *Dermacentor reticulatus* and *Ixodes ricinus* ticks are found mainly in grass, not only in forested areas but also in urban parks, with tick densities under forest cover being 7.1 times higher than in a suburban park (Mathews-Martin et al., 2020).

Babesiosis is considered a seasonal disease, but its disappearance, regardless of the season, correlated with a sharp decrease in temperature ($\Delta T > 9°C$) in combination with heavy rain, prolonged drought, or temperatures above 20°C in spring and summer or below 5°C in autumn and winter (Leschnik et al., 2008). To maintain an epizootic outbreak of babesiosis, three links are necessary: infected or previously ill animals, vector ticks, and susceptible animals (Young et al., 2019). Today, with the spread of ectoparasite habitats, the increase in the number of stray animals in cities and other settlements, and the preservation of a large number of susceptible dogs, all three links in the epizootic chain remain active, which contributes to the further growth of natural outbreaks and transmissible diseases, including babesiosis (Adaszek et al., 2012). *Babesia bovis* is a much more dangerous organism than *B. bigemina*, since in most of its strains, pathogenic effects are largely due to the destruction of erythrocytes (Peixoto et al., 2022).

Four species of *Babesia* cause canine babesiosis (*B. canis*, *B. rossi*, *B. vogeli*, and the unofficial *B. coco*). While canine babesiosis is widely distributed worldwide, each species has a specific geographic range: *B. rossi* is found in sub-Saharan Africa, *B. canis* in Europe and Asia, *B. coco* in the eastern Atlantic of the United States, and *B. vogeli* is distributed in Africa, southern Europe and Asia, northern Australia, southern regions of North America, and South America. Of all these species, *B. vogeli* is the most widespread globally (Zygner et al., 2023). A total of 971 ticks were collected at nine locations in Kyiv and the Kyiv region, the largest and most densely populated metropolis in Ukraine, including *Ixodes ricinus* (60.5%), *Dermacentor reticulatus* (39.4%), and a single male *Rhipicephalus sanguineus* sensu lato (0.1%, Rogovskyy et al., 2017).

The study aims to determine the prevalence of *Ixodes ricinus* ticks infected with *Babesia* spp. and to assess the epizootiological characteristics of canine babesiosis distribution in various districts of the Kharkiv region and the city of Kharkiv during 2012-2023, taking into account seasonal dynamics, age-related susceptibility of dogs, and conditions of the urban environment.

MATERIALS AND METHODS

Ethical approval

Experiments conducted on animals do not contradict the materials of the IV European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes (Simmonds, 2018). The study was reviewed and approved by the Bioethics Commission of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" in accordance with the current procedure.

Tick collection and identification

Studies to establish the role of the arthropod *I. ricinus* in the spread of vector-borne diseases such as babesiosis were conducted in two stages from 2012 to 2016 and from 2017 to 2023 in connection with the administrative-territorial reform, which resulted in the unification of districts, which led to the regrouping of acarological monitoring zones.

Acarological collections were conducted from 2012 to 2016 in natural biotopes of nine districts of the Kharkiv region (Kupyansky, Dvurichansky, Vovchansky, Bogodukhivsky, Izyumsky, Balakliysky, Zmiivsky, Chuguyivsky, Kharkivsky). The total area of the studied territory was 15.54 thousand km^2, which is 49.5% of the area of the Kharkiv region (Figure 1). From 2017 to 2023, archaeological collections were conducted in natural centers of four districts of the Kharkiv region (Kharkiv, Izyum, Velykoburlutsky, Chuhuyiv, Figure 2). The survey area was 18.55 thousand km^2, which is 59.1% of the area of the region. During 2021-2023, monitoring studies of *Ixodes ricinus* infestation were conducted in the districts of Kharkiv (Osnovyansky, Kyivsky, Kholodnohirsky, and Oleksiyivsky [Shevchenkivsky], Figure 3).

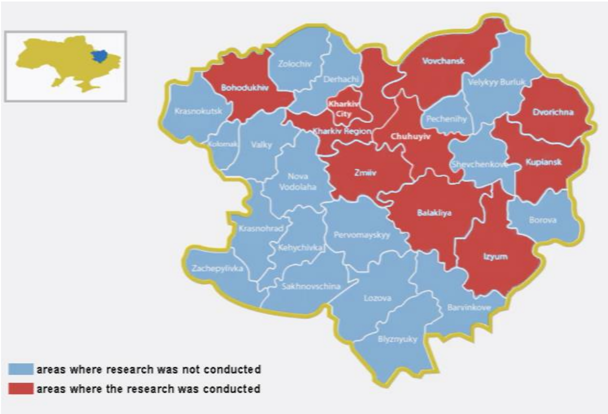


Figure 1. Districts of Kharkiv region, Ukraine, where arachnological collections were conducted (2012-2016)

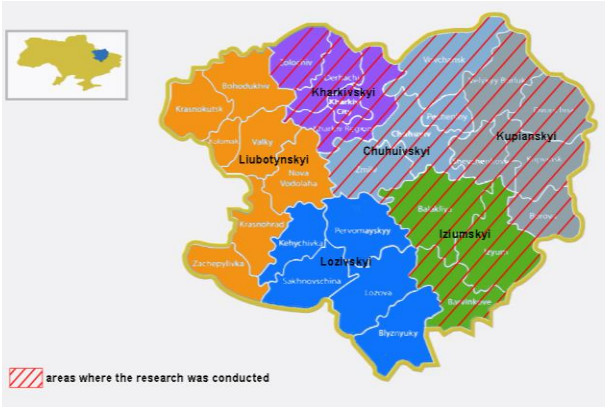


Figure 2. Districts of Kharkiv region, Ukraine, where arachnological collections were conducted (2017-2023)

Figure 3. Districts of Kharkiv, Ukraine, where a carological collections were conducted (2021-2023)

A total of 2,690 *Ixodes ricinus* ticks were collected from natural habitats, of which 1,955 were from Kharkiv region (544 nymphs and 1,411 adults) and 735 from Kharkiv city (11 nymphs and 724 adults, as well as 294 from dogs. Ticks were collected in the park and residential areas on routes of equal distance (2 km), on the same dates (10-20 May), using a blanket of fluffy white fabric (60 × 100 cm), using both drag and flagging. Ticks were removed from the blanket with tweezers and placed in plastic containers 10 cm long and 3 cm in diameter with holes in the lid. A strip of sterile paper moistened with water was previously placed in the container (Salomon et al., 2020; Lyons et al., 2021).

Tick removal was performed with a special hook, carefully grabbing the tick as close to the dog's skin as possible. The tick was slowly and smoothly pulled upwards, trying not to twist it. Ticks collected from animals were placed in Petri dishes on wet paper. Then, the developmental stage, genus, and species of each tick were identified through visual and microscopic examination using established morphological keys, which included the length and shape of the capitulum, the shape and pattern of the scutum, and the absence of eyes (Ernieenor et al., 2017; Estrada-Peña et al., 2017). Polymerase chain reaction (PCR) analysis was not conducted in this study. The determination of the tick species was carried out in the laboratory of veterinary sanitation, parasitology, and the study of bee diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv, Ukraine). Since 2011, no acaricidal treatments have been applied in the natural biocenoses of Kharkiv Oblast and the city of Kharkiv, allowing these areas to be considered free from anthropogenic impact on tick populations.

Detection and analysis of babesiosis

A total of 1,486 dog blood samples were tested, of which 578 were from the Kharkiv region and 908 were from the city of Kharkiv. *Babesia* was detected by analyzing thin blood smears obtained from the ear veins of dogs. The smears were fixed in methanol for 3 minutes, stained with Romanowski-Giemsa solution for 25 minutes, and then rinsed with distilled water (Urquhart et al., 1996). Microscopic examination was performed using an Axioskop 40 microscope (Zeiss) equipped with a Jenoptik Laser Optic System D-07739 digital camera (Germany, 2008) under oil immersion at 1000× magnification. Samples were considered positive if single trophozoites or paired pear-shaped merozoites of *Babesia* sp. were detected in erythrocytes. For morphometric analysis, positive samples were selected to perform comparative measurements of parasite size. All studies were conducted in the Laboratory of Veterinary Sanitation, Parasitology, and Bee Disease Investigation of the National Scientific Center "IEKVM" (Kharkiv, Ukraine).

Statistical analysis

The extent of infestation (EI) was determined by the following formula.

$$EI = X/Y \times 100$$

Where X is the number of animals (ticks) in which *Babesia* was found, Y is the total number of examined animals (ticks), and 100 is the conversion factor into percentage.

RESULTS

The study's results showed that the infestation of *Ixodes ricinus* ticks with *Babesia* in natural foci of the Kharkiv region is not stable. In total, 1171 specimens of *Ixodes ricinus* ticks were examined over five years, of which 793 (67.7%) were infected with *Babesia* (Table 1). In 2012, the extent of *Babesia* infestation of *I. ricinus* ticks was 72.3%, in 2013 it was 66.2%, in 2014 it was 52.6%, in 2015 it was 81.1%, and in 2016 it was 74.6%. The lowest *Babesia* infestation of *I. ricinus* nymphs (25.8%) and imago (26.8%) was recorded in 2014. The average infestation rate for 5 years of *I. ricinus* nymphs was 33.3 ± 7.9%, and imago 36.0 ± 5.5%, which is an indicator of transphase infection of ticks with the causative agent of babesiosis and the endemicity of the disease in this region.

The number of males bitten by ticks relative to the number of females was 1.21:1 (317 males and 261 females). *Ixodes ricinus* mites were most frequently recorded in dogs aged 5-6 years (201 [34.8%]), somewhat less frequently in animals aged 2-4 years was (179 [30.9%]), 7-9 years was(162 [28.0%]), puppies aged 6 months–1 year was(24 [4.2%]), and least frequently in dogs aged 10 years and older was(12 [2.1%]). The average extent of *Babesia* infection in dogs during the study period was 36.5%. The lowest prevalence of *Babesia* infection in dogs was recorded in 2014 (31.5%, Table 2). Over seven years, 784 specimens of *I. ricinus* ticks were examined, including 617 imago and 167 nymphs. Of these, 541 (57.5%) were found to be infected with babesiosis pathogens. Infection was detected in all nymphs examined, as well as in 284 imagoes, which is 46.0% of the total number of imagoes (Table 3). In 2020 and 2021, no *Ixodes ricinus* nymphs were detected, likely due to extremely dry conditions (annual precipitation was less than 279 mm). In these years, the average level of *Babesia* infection was 21.3% among nymphs and 36.2% imago adults. Compared to 2012–2016, nymph infection was 10.9% lower, while imago infection increased by only 0.4%. During the period from 2017 to 2023, 908 blood samples from dogs bitten by *I. ricinus* ticks were examined from the studied districts of the Kharkiv region. The average extent of *Babesia* infection in dogs was 37.2%. The lowest prevalence of *Babesia* infection in dogs was recorded in 2022 and 2023 (34.1%, Table 4).

Table 1. Infestation of *Ixodes ricinus* ticks with *Babesia* in natural foci in the Kharkiv region, Ukraine (2012-2016)

Year	Examined ticks	Infected nymphs	EI (%)	Infected imago	EI (%)
2012	256	80	31.3	105	41.0
2013	231	61	26.4	92	39.8
2014	306	79	25.8	82	26.8
2015	185	81	43.8	69	37.3
2016	193	76	39.4	68	35.2

EI: Extent of infestation

Table2. The blood samples of infected dogs with *Babesia* after *Ixodes ricinus* attacks in Kharkiv region, Ukraine (2012-2016)

Year	Blood samples	Positive samples	EI (%)
2012	128	57	44.5
2013	105	39	37.1
2014	146	46	31.5
2015	98	35	35.7
2016	101	34	33.7

EI: Extent of infestation

Table3. Infestation of *Ixodes ricinus* ticks with *Babesia* in natural foci in the Kharkiv region, Ukraine (2017-2023)

Year	Examined ticks	Infected nymphs	EI (%)	Infected imago	EI (%)
2017	112	33	29.5	39	34.8
2018	105	36	34.3	40	38.1
2019	106	37	34.9	42	39.6
2020	105	–	–	37	35.2
2021	112	–	–	38	33.9
2022	120	30	25.0	43	35.8
2023	124	31	25.0	45	36.3

EI: Extent of infestation

Table4. The blood samples of infected dogs with *Babesia* after *Ixodes ricinus* attacks in the Kharkiv region, Ukraine (2017-2023)

Year	Blood samples	Positive samples	EI (%)
2017	121	61	50.4
2018	143	53	37.1
2019	125	44	35.2
2020	131	47	35.9
2021	124	43	34.7
2022	135	46	34.1
2023	129	44	34.1

EI: Extent of infestation

The decrease in the prevalence of *Babesia* spp. in dogs may be due to the use of modern drugs (Bravecto, Merck Animal Health, New Jersey, USA; Simparica, Zoetis Inc., New Jersey, USA) to protect animals from tick infection. The number of males bitten by ticks relative to the number of females was 1.41:1 (532 males and 376 females).

Ixodesricinus mites were most frequently recorded in dogs aged 5-6 years (357 [39.3%]), less in animals aged 2-4 years (246 [27.1%]), 7-9 years (213 [23.5%]), puppies aged 6 months-1 year (56 [6.2%]), and least in dogs aged 10 years and older was (36 [3.9%]).

The decrease in dog infection with *Babesia* spp. can be explained by the use of new forms of protection against tick infestation. In 2021, out of 318 specimens of *I. ricinus* collected "on a flag" from natural biotopes of Kharkiv, 40.6% were infected with *Babesia* spp. The most infected were ticks collected in the Oleksiyivskyi (Shevchenkivskyi) district of Kharkiv, 57.8% (48 out of 83, Table 5). In 2022, out of 221 specimens of Ixodes ricinus, 106 ticks (47.9%) were infected, and in 2023, out of 196 specimens of *I. ricinus*, 97 specimens (49.5%) were infected.

In Shevchenkivskyi district, the infestation of *I. ricinus* mites decreased in the park area, but increased in the residential area, probably due to the growth of development in the district. A similar trend was observed in all districts of the city. Despite a decrease in the number of studied ticks in 2021-2023, their average infestation increased by 8.9% in 2023 compared to 2021. Therefore, the probability of infection of dogs, both when staying in the park area and the residential area, was increased.During the examination of 152 dogs from Kharkiv, 294 ticks were removed, of which 154 (52.4%) *I. ricinus* were infected with *Babesia*. Of the animals bitten by ticks, 46.1% developed babesiosis (Table 6).It was found that in Kharkiv, the number of males bitten by ticks relative to the number of females was 1.3:1 (87 males and 65 females).

The most dogs with suspected suspected babesiosis was registered at the age of 5-6 years (77 [50.7%]), followed by animals 2-4 years old (39 [25.7%]), 7-9 years old (28 [18.4%]), older than 10 years old (5 [3.3%]), and the smallest number was registered in puppies aged 6 months to one year (3 [1.9%]). Among dogs infected with babesiosis, dogs aged 10 and older accounted for 1.4%, puppies were 4.3%, dogs aged 5-6 years were 51.4%, and other age groups were 42.9%.When determining the seasonal dynamics of canine babesiosis in Kharkiv, two peaks of incidence were established in April-May (18.3-22.7%) and September (16.1%, Graph 1). The number of sick males relative to the number of females was 1.5:1.

Table 5. Infestation of *Ixodes ricinus* ticks with *Babesia* in districts of Kharkiv city, Ukraine (2021-2023)

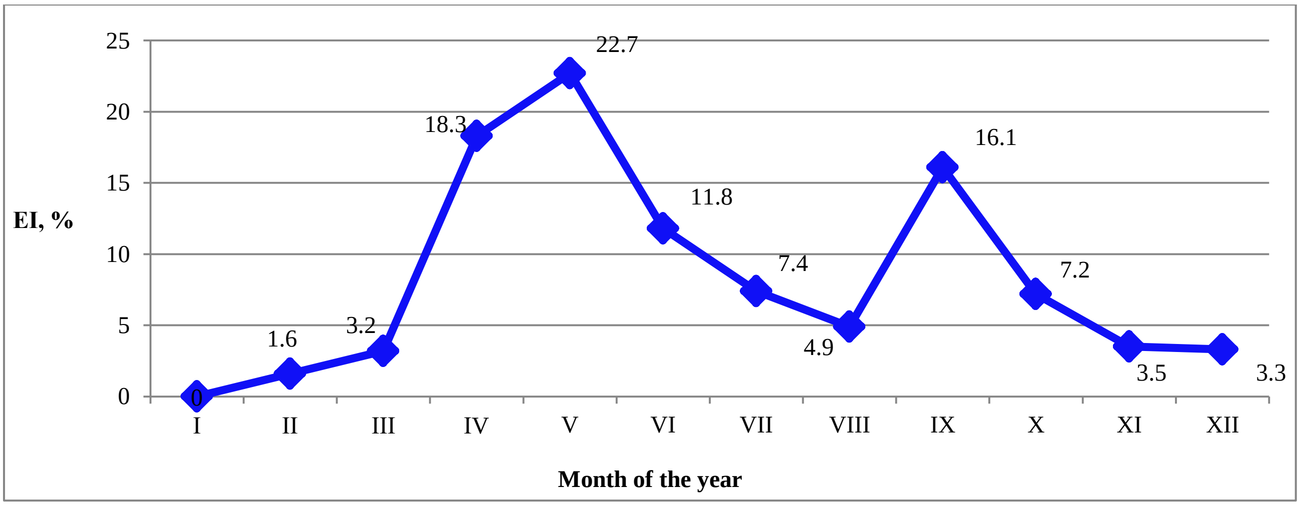
Year	Districts	Park area			Residential area		
		Examined ticks	Infested ticks	EI (%)	Ticks were examined, specimens	Infested ticks	EI (%)
2021	Osnovyansky	45	21	46.7	48	12	25.0
	Kyivsky	43	18	41.9	38	6	15.8
	Kholodnohirsky	42	19	45.2	20	6	30.0
	Oleksiyivsky	55	34	61.8	28	14	50.0
Total		**185**	**92**	49.7	**133**	**37**	27.8
2022	Osnovyansky	31	14	45.2	22	10	45.5
	Kyivsky	29	13	44.8	21	8	38.1
	Kholodnohirsky	32	15	46.9	22	12	54.5
	Shevchenkivsky	40	21	52.5	24	13	54.2
Total		**132**	**63**	47.7	**89**	**43**	48.3
2023	Osnovyansky	29	11	37.9	20	9	45.0
	Kyivsky	25	14	56.0	15	7	46.7
	Kholodnohirsky	30	16	53.3	19	11	57.9
	Shevchenkivsky	37	18	48.6	21	11	52.4
Total		**121**	**59**	48.8	**75**	**38**	50.7

EI: Extent of infestation

Table 6. *Babesia* infection of *Ixodes ricinus* ticks removed from dogs from Kharkiv city, Ukraine (2021-2023)

Year	Districts	Examined animals	Examined ticks	Infested ticks	EI (%)	Sick animals (Number)
2021	Osnovyansky	18	28	16	57.1	8
	Kyivsky	10	23	10	43.5	5
	Kholodnohirsky	16	36	14	38.8	7
	Oleksiyivsky	19	49	27	55.1	16
Total		**63**	**136**	**72**	**52.9**	**36**
2022	Osnovyansky	15	23	12	52.2	5
	Kyivsky	14	16	9	56.3	5
	Kholodnohirsky	7	19	9	47.4	4
	Shevchenkivsky	12	27	14	51.9	5
Total		**48**	**85**	**44**	**51.8**	**19**
2023	Osnovyansky	15	25	12	51.8	5
	Kyivsky	10	11	6	54.5	3
	Kholodnohirsky	7	12	6	50.0	3
	Shevchenkivsky	9	25	14	56.0	4
Total		**41**	**73**	**38**	**52.1**	**15**

EI: Extent of infestation



Graph 1. Dynamics of the incidence of babesiosis in dogs in the city of Kharkiv, Ukraine (2021-2023)

DISCUSSION

The unicellular parasite *Babesia canis*, which infects erythrocytes, is a common hemoparasite transmitted by *Ixodes* ticks and poses a threat to the health and life of dogs worldwide (Hildebrandt et al., 2020). The trend towards the spread of vector-borne diseases is quite threatening (Zhabykpayeva et al., 2023). Control of the number of parasitic insects and ticks should be based on scientifically sound approaches, taking into account the eco-geographical characteristics of the regions (Boulanger et al., 2024). In Europe, studies have indicated that the infection rate of *Ixodes ricinus* nymphs with *Babesia* has averaged between 0 and 100% (Blaschitz et al., 2008). In the United Kingdom (UK), 0.38% of *I. ricinus* nymphs were found to be infected with *Babesia* spp. (Gandy et al., 2024). Only 1% of *I. ricinus* nymphs were infected with *B. divergens*, which corresponds to the infection rates observed in ticks collected on farms and in forests of Ireland, where cattle grazing was restricted until the early 1990s due to the widespread prevalence of redwater fever, leading to a decline in *I. ricinus* and *B. divergens* populations (McKiernan et al., 2022). In Kharkiv, the level of greening of the city is 50.8%. The total area of the city territory is 30,604.0 hectares, including the area of green spaces were 15,407.0 hectares. Green spaces in the city occupy 31 parks, 5 gardens, 150 squares and boulevards, and over 500 greened corners. Green spaces are represented by broad-leaved tree species, while acaricidal agents for tick control in parks and residential areas are not used (Perepelytsia, 2020). In natural foci of the Kharkiv region, all nymphs and 46% of adults of the *Ixodes ricinus* tick were found to be infected. Within the city, infection rates also remain high: in the park area, the infection rate of nymphs is 51.1%, and in the residential area, 39.7%. The reduction in the number of ticks in the park zone of the city of Kharkiv is associated with an improvement in the quality of vegetation maintenance. Preservation of natural ecosystems during development preserves biodiversity but leads to an increase in the spread of Ixodes ticks in the residential area.

According to previous studies, the incidence of babesiosis in dogs depends on age. Puppies under three months of age rarely get sick, the highest rates are recorded in animals from one to three years old, and in older age groups the prevalence decreases slightly, which is explained not by age-related immunity, but by a lower probability of contact with vector ticks (Obeta et al., 2020; Janjić et al., 2024). Among dogs tested for babesiosis, the highest incidence was found in the age group of 5-6 years (51.4%), while the lowest number of infected animals was recorded in the group older than 10 years (1.4%), which may be due to a decrease in susceptibility to the disease with age, as well as the formation of immunity upon repeated infections.

Although no precise quantitative data are available regarding the proportion of dogs that received acaricidal treatments during 2017-2023, the observed decline in *Babesia* spp. Infection among dogs may be partially attributed to the increased use of modern isoxazoline-based tick protection products (e.g., fluralaner–Bravecto, sarolaner–Simparica), which have demonstrated high efficacy against *I. ricinus* and have become widely accessible to pet owners. The use of acaricidal treatments is a key preventive strategy that contributes to reducing the risk of vector-borne infections, including babesiosis, in dogs (Paliy et al., 2022; Dubova et al., 2023). Furthermore, studies conducted in Bila Tserkva emphasized the importance of seasonal prevention using anti-tick agents to lower infection rates during peak tick activity in spring and autumn (Antipov et al., 2018).

Babesiosis has a pronounced seasonality, which correlates with the activity of the Ixodes tick vector, as confirmed by many years of research. In France, *Dermacentor reticulatus* causes cases of piroplasmosis in spring and autumn during the periods of its imago activity (René-Martellet et al., 2015). Analysis of the seasonal dynamics of babesiosis in dogs in Kharkiv (Ukraine), which is located in the transition zone from forest-steppe to steppe, revealed two pronounced peaks of incidence: in spring (April-May), 18.3-22.7% and in autumn (September),16.1%. Such indicators correlate with periods of increased activity of Ixodes ticks within the urban environment, where, as noted earlier, acaricidal agents are not used, and the level of greenery exceeds 50%. This creates favorable conditions for maintaining the population of vectors and, accordingly, preserving the natural focal nature of the invasion.

Studies conducted in the central part of Ukraine, which mainly belongs to the forest-steppe zone, revealed a characteristic seasonal dynamics of the incidence of babesiosis in dogs. The main peak fell in May, when the infection reached almost 30% of the examined animals. The second, less pronounced peak was observed in September–October, accounting for about 6%. In the winter period, no cases of babesiosis were recorded, which corresponds to the seasonal biological inactivity of Ixodes ticks (Antipov et al., 2018). In the territory of Polissya in Ukraine, outbreaks of acute babesiosis in dogs coincide with periods of Ixodes tick activity, mainly recorded in spring (March-June) and autumn (late August-November; Dubova et al., 2023).

CONCLUSION

Both males and females were equally susceptible to babesiosis, with the ratio of tick-bitten males to females being 1.33:1 in the study region. Babesiosis in dogs is detected throughout the year, but has a pronounced seasonality with two peak periods. The results of epizootological monitoring indicate that the issue of babesiosis control in the Kharkiv region and the city of Kharkiv remains relevant. In this regard, there is a need to develop and implement scientifically based preventive measures aimed at reducing the incidence of dog bites. Therefore, future scientific research should prioritize epidemiological monitoring of *Babesia* spp. distribution across different regions and evaluate the impact of climate change and environmental factors on vector population dynamics.

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Competing interests

The authors have not declared any conflict of interest.

Authors' contributions

Mykola Bogach and Anatoliy Paliy participated in the data collection, analysis, preparation, and revision of the manuscript. Natalia Sumakova, Lilia Roman, Olena Bohach, and Anatoliy Kiptenko contributed to the data collection and laboratory analysis, while Olena Pavlichenko and Denis Bohach formatted and edited the manuscript. All authors read and approved the final manuscripts.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript. Additional datasets are available upon reasonable request from the corresponding author.

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