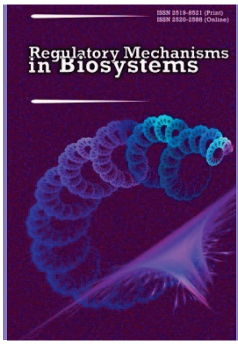

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Influence of ecological and climatic conditions on the spread of *Borrelia burgdorferi* in domestic dogs in Ukraine

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Lyme-borreliosis is a zoonotic, infectious disease that has a complex chain of transmission of the pathogen *Borrelia burgdorferi sensu lato* and includes the relationship between ixodid ticks, vertebrate hosts, humans and companion animals in the environment. The article shows general trends in the prevalence of canine Lyme-borreliosis in Ukraine depending on environmental, climatic and physiographic factors. The results of a comparative cartographic analysis of the prevalence of Lyme borreliosis among domestic dogs in Ukraine are presented by systematizing, mathematical and statistical processing of the data obtained by surveying veterinarians engaged in clinical veterinary practice. The paper includes generalized data on the clinical manifestations, methods of diagnosis and treatment of Lyme borreliosis in dogs. We determined the dependence of the prevalence of Lyme borreliosis in dogs on the types of physical and geographical territories – natural zones of Ukraine. Each of the natural zones differs in types of relief, climatic conditions, soil types, composition of fauna and flora, which affect the epizootic chain of Lyme disease. There is a clear correlation between the incidence of Lyme borreliosis in dogs and the types of natural areas. The highest incidence of Lyme borreliosis in dogs was observed in the forest-steppe zone and the zone of broad-leaved forests. A sharp decrease in the incidence of dogs was recorded in areas of mixed forests, the Ukrainian Carpathians and in the South of Ukraine in the steppe zone. The study also confirmed that the prevalence of Lyme disease among domestic dogs was influenced by the climatic factors, in particular: gross moisture of territories, average annual air temperature and soil temperature. In Ukraine, veterinarians in the vast majority of cases use serological diagnostic methods: immunochromatographic analysis, immunoenzymatic assay, and western blot, which are insufficient, since the presence of antibodies to the Lyme borreliosis pathogen is only a confirmation of the animal's contact with the antigen and may not indicate the presence of the disease in the clinical form. The generalized data on the use of antimicrobial drugs in the treatment of Lyme borreliosis in dogs indicate the predominant use of tetracycline antibiotics and cephalosporins. The majority of veterinarians reported symptoms of Lyme arthritis, somewhat fewer reported Lyme nephritis, neuroborreliosis, Lyme carditis and in rare cases, veterinarians observed erythema at the site of tick bite. About half of the veterinarians in Ukraine observed an increase in the incidence of Lyme disease in dogs, indicating a probable deterioration of the epizootic and epidemiological situation regarding Lyme borreliosis, especially in areas with favourable conditions for the circulation of Lyme borreliosis pathogens in natural and urban ecotopes. The results substantiate the need for the further study of the circulation of *Borrelia burgdorferi sensu lato* and their ability to cause disease in humans and animals, as well as the need to implement the principles of the One Health concept for the control and management of Lyme borreliosis.

Keywords: tick-borne disease in dogs; ecology of Lyme borreliosis; *Borrelia burgdorferi*; *Ixodes ricinus*; *Ixodes persulcatus*; *Dermacentor marginatus*; *Rhipicephalus sanguineus*; *Hyalomma marginatum*.

Introduction

Lyme borreliosis (synonyms: Lyme disease, borreliosis, ixod tick-borne borreliosis, LB) is a zoonotic, transboundary, obligately transmissible, infectious disease of a natural focal nature, which imposes a significant socio-economic and veterinary-practical burden on the countries of the Northern Hemisphere. Lyme disease is widespread in North America and European countries with temperate climate, including Ukraine (Feria-Arroyo et al., 2014; Strnad et al., 2017; Rogovskyy et al., 2020).

The infection agent of LB is a bacterium that belongs to the family Spirochaetaceae, genus *Borrelia*, species *Borrelia burgdorferi*, which has about 21 genotypes (Cutler et al., 2017). Transmission of Bb occurs through ticks of the Ixodidae family, mainly the species *Ixodes ricinus*, *I. hexagonus*, *I. persulcatus* and, to a lesser extent, other species of hard-bodied ticks: *Dermacentor reticulatus*, *D. marginatus*, *Rhipicephalus rossicus*, *R. sanguineus*, *R. bursa*, *Hyalomma punctata* and *H. marginatum*. Dozens of small mammals, some species of lizards, and birds are their reservoir hosts. In the Bb transmission cycle, humans and companion animals become accidental hosts (Biletska et al., 2008; Levytska & Mushynskyi, 2020; Nebogatkin & Shulhan, 2020). As observed by scientists, the geographic area of ixodid ticks is expanding due to environmental factors, especially the climate change and anthropogenic impact on biotopes, creating an additional risk of infection of humans and animals with Lyme borreliosis (Medlock et al., 2013; Springer et al., 2020; McVicar et al., 2022).

Since 2000, the Ministry of Health of Ukraine (MOH) has been recording cases of Lyme disease in Ukraine. Every year, the Public Health Center of the MOH reports on the number and frequency of LB cases among the population. The incidence of LB is noted throughout the territory of Ukraine. The most cases are registered in Kyiv (29.00 cases per 100, 000 population), Cherkasy (25.4), Vinnytsia (23.9), Sumy (25.89) regions, and the city of Kyiv (22.54) (Chemych & Lutai, 2020; Nebogatkin & Shulhan, 2020). In dogs, LB has been studied in-depth by veterinarians in the United States. For example, in 2018, there was an update of the consensus of the American College of Veterinary Internal Medicine (ACVIM), whose task was to summarize the knowledge about LB in animals. The need to screen clinically healthy dogs for seroprevalence of Bb living in and near-endemic areas has been confirmed. Although the detection of antibodies to Bb indicates exposure to the antigen, it does not guarantee the development of clinical signs of LB (Littman et al., 2018). In Ukraine, the information regarding aspects of the distribution of LB among animals is limited and insufficient to understand the epizootic situation (Panteleienko et al., 2021).

In the scientific literature, most clinical cases of LB are described in dogs, although there are scientific publications about cases of the disease in other species of animals, for example, horses and cats (Janus et al., 2014; Divers et al., 2018; Hoyt et al., 2018). In most cases, animals seropositive for *B. burgdorferi* may not have LB clinically, but, nevertheless, the clinical signs of Lyme disease are quite diverse. Depending on which organs and body systems are affected by Borrelia, those signs are divided into certain groups (Littman et al., 2018b; Soroka et al., 2019). Lyme arthritis is observed, which manifests itself as oligoarthritis or polyarthritis, while in the area of the joints, pain, swelling and an increase in local temperature occur, as well as possible lymphadenopathy and fever. Lyme nephritis is a nephropathy that is associated with damage to the kidney glomeruli – glomerulonephritis with proinuria, possible development of nephrotic syndrome, and sometimes, lameness is observed (Littman et al., 2006). Neuroborreliosis is manifested by astasia, tonic convulsions and hyperreflexia (Azuma et al., 1993; Imai et al., 2011; Johnstone et al., 2016). Lyme carditis usually presents with an atrioventricular conduction disorder (Raveche et al., 2005; Janus et al., 2014; Adaszek et al., 2020). It is believed that the different antigenicity of *B. burgdorferi* strains circulating in different continents and territories may explain the differences in the clinical signs observed in dogs in the USA, Europe and Japan (Azuma et al., 1993).

Currently, scientists and veterinary practitioners have no single approach to the diagnosis of LB in animals. Oftentimes, in practice, confirmation of the LB diagnosis in animals is based on the serological diagnosis. However, the studies indicate that the presence of antibodies against *B. burgdorferi* surface proteins is not always the cause of clinical signs (Wagner et al., 2012; Callister et al., 2015; Littman et al., 2018a; Fudge et al., 2020). A more informative method of diagnosis is PCR assay of skin biopsy samples of the site of tick attachment, synovial samples from affected joints, and additional examination of the tick that had been feeding on the animal (Rijpkema et al., 1997; Wilske et al., 2007).

The treatment of LB in animals consists of antimicrobial medicine and additionally immunosuppressive medicine for Lyme nephritis. According to the recommendations of the ACVIM consensus statement (Littman et al., 2018a), it is best to use antibiotics from the tetracycline group – doxycycline or minocycline in the dose of 5–10 mg/kg orally, every 12 hours, for 4 weeks. Usually, LB symptoms disappear 1–3 days after starting antibiotic therapy, but if this does not happen, other diagnoses should be considered, such as immune-mediated polyarthropathy. Currently, there is no definitive protocol for the treatment of LB (Ettinger et al., 2017).

Since there are differences in the population of ticks in different territories of Ukraine and the infections they carry, we assume that there is a relationship between the frequency of LB cases in animals, geographical areas, and climatic features, which, in turn, may have an impact on the epizootic situation regarding LB animals in different regions. The aim of our study was to determine whether physical-geographical and climatic factors have an influence on the prevalence of LB in animals, how often veterinarians encounter LB, and their opinions about the trend of increasing incidence of LB in different geographical areas. To determine which clinical signs of LB in animals are most common and determine the most effective methods of diagnosis and treatment of LB, we analyzed the experience of veterinarians and the data they provided us.

The objective of our study was the influence of physical, geographical, and climatic factors on the prevalence of LB in animals, the frequency of diagnosis of LB by veterinary specialists, to clarify the prerequisites for the development of a tendency of increasing incidence of LB in different geographical areas and to determine the commonest clinical signs and methods of diagnosis and treatment of LB in animals, based on the experience and data provided by veterinarians. At this moment, it is unknown how widespread LB is in animals in Ukraine and what approaches are used by veterinarians in the fight against LB, so a better understanding would help lead further directions of research and determine strategies for the elimination of LB.

Materials and methods

Our research consisted of four consecutive stages. At the first stage, in order to collect the data on LB in domestic animals, we conducted an online survey among Ukrainian veterinarians using Google Forms. The questionnaire form contained multiple-choice questions and an additional line to allow a respondent to self-report additional information. The questions were related to the data about the administrative regions in which the respondents provide veterinary services; about the experience of clinical practice of the respondents; about how many cases of LB in animals a respondent has recorded and in which species; about the clinical LB manifestations that were observed; about the methods of diagnosis and treatment of LB-infected animals. We were also interested in the opinion of practicing veterinarians regarding the dynamics of recording LB cases in animals, whether they have observed a trend towards its increase. Google Forms is always open for monitoring, filling and feedback via the link http://surl.li/ebugc. The questionnaires were sent to 340 veterinarians working with small animals in veterinary hospitals of Ukraine. Completed questionnaires were received from 285 veterinarians from different regions of Ukraine (Table 1).

Table 1
Information on the number of questionnaires on Lyme borreliosis in animals received from veterinarians by regions of Ukraine

Region of Ukraine	Number of completed questionnaires of veterinarians from the region
Vinnytsia	9
Volyn	9
Dnipropetrovsk	14
Zhytomyr	16
Transcarpathian	8
Zaporizhzhya	11
Ivano-Frankivsk	13
Kyiv	24
City of Kyiv	31
Kirovograd	9
Lviv	11
Mykolaiv	12
Odesa	9
Poltava	7
Rivne	8
Sumy	12
Ternopil	9
Kharkiv	16
Kherson	11
Khmelnytsky	10
Cherkasy	17
Chernivtsi	9
Chernihiv	10

At the second stage, structuring and mathematical processing of the received data were carried out. Respondents' answers were divided into five groups: Group 1 included data on the region of the respondents' service area, the name of the settlement of the respondents' service area, the respondents' work experience (number of years), the number of diagnosed cases of LB in animals during their period of work; Group 2 – information on diagnostic methods for LB in animals, Group 3 – clinical signs of LB in animals, Group 4 – use of antimicrobial drugs in the treatment of LB in animals, and Group 5 – observations of respondents regarding the dynamics of the frequency of LB cases in animals. By the means of mathematical data processing, we determined the average number of LB cases in animals per year in the form of the mean for each respondent, then separately for settlements and across the regions of Ukraine for the data of Group 1.

At the third stage, we performed a cartographic analysis using the free software QGIS 3.4.6 (USA, 2019). The choropleth maps were created using projection CRS:EPSG:102013 Europe Albers Equal Area Conic. Vector layers of the borders and regions of Ukraine were downloaded from the resource with open spatial data Vector layers of Ukraine's borders and regions were obtained using free spatial data Diva-Gis (www.diva-gis.org/Data). The obtained results of calculations of the average number of LB cases in animals per year were geo-coordinated (Retrieved from https://dateandtime.info/uk/citycoordinates.php) and plotted on a map per region of Ukraine. Taking into account that climatic factors and physical-geographic differences of territories can indirectly affect the prevalence of LB, we added geo-coordinated data of calculations of the average number of LB cases in animals per year on maps of Ukraine with separate climatic indicators, such as the average temperature of the soil surface in the warm time of the year (in July), gross soil moisture (difference between precipitation and surface runoff), average air temperature for the year (range of observed air temperatures throughout the year), as well as on the map with natural zones: zone of mixed forests, zone of broad-leaved forests, forest-steppe zone, the Ukrainian Carpathians, the steppe zone, the Crimean Mountains and the Crimean South Coast zone (https://geomap.land.kiev.ua/climate.html). It should be noted that the zones of individual climatic indicators and natural zones do not have clear boundaries, but smoothly merge into one another. Therefore, if a geo-coordinated indicator was located on the border of climatic/ natural zones, we attributed it to both bordering zones. Also, if answers had been received from several respondents from one settlement, city, or village, their data were combined, the mean was calculated, and the geo-coordinated average number of LB cases in animals per year was shown on maps.

The fourth stage was the statistical data analysis. The following descriptors were used to characterize the data groups: mean (x), standard deviation (x ± SD), median, minimum (min) and maximum values (max). The normality of the frequency distribution of each group of the data was verified using the Shapiro-Wilk Test. We aimed to make a comparison between the groups divided by certain climatic indicators and natural zones of Ukraine and to determine whether climatic factors affect how often veterinarians encounter LB in animals. The dependent variable was the average number of LB cases in animals, the independent variable was the zones of individual climatic indicators and natural zones of Ukraine. Free software Jamovi, version 1.6 (Australia, 2021, www.jamovi.org) was used for the statistical analysis. The statistical analysis of data was carried out by a non-parametric test with one-way variance analysis of ranks – Kruskal-Wallis test. The results were considered statistically significant if $P < 0.05$.

Results

As a result of the survey among veterinarians of Ukraine, 285 completed questionnaires were received. The information was received from almost every region of Ukraine, except Luhansk, Donetsk regions and the Autonomous Republic of Crimea, and the city of Sevastopol, as these territories are temporarily occupied by the Russian Federation at the time of the survey. The largest number of answers was received from the city of Kyiv and the Kyiv region, 10.9% and 8.4%, respectively, out of all 285 processed questionnaires. The fewest responses came from Zakarpattia, Rivne, and Poltava regions, 2.5–2.8%, respectively. In all other regions of Ukraine, the percentage of respondents ranged from 3.2% to 6.0%. The majority of the respondents had 5 to 15 years experience of clinical practice (71.6%), fewer have worked 1 to 5 years (16.8%), and veterinarians with 15 or more years of experience gave the least feedback (11.6%).

In 11 of 285 submitted questionnaires, the veterinarians indicated that they had never encountered cases of LB in animals in their practice, namely: these are four veterinarians from the Kherson region, two each from Mykolaiv and Zaporizhia regions, and one each from Kharkiv, Lviv, and Ivano-Frankivsk regions, respectively. In the case of the last three regions, the clinical experience of doctors did not exceed 2.5 years. It should also be noted that all other 274 respondents who diagnosed and treated LB reported that clinical cases were noted only in dogs, so we shall describe LB only in this species of animal.

As a result of calculations of the average number of cases of LB in dogs per year in the administrative regions of Ukraine, we obtained the following data: the highest average indicator – 1.57 cases of LB per year was observed in Chernihiv region; in Kyiv, Cherkasy, Sumy, Khmelnytskyi, Vinnytsia, Kharkiv, Lviv, and Ternopil regions, the indicator ranged 0.97 to 1.45 cases of LB per year; in Zhytomyr, Kirovohrad, Dnipropetrovsk, and Rivne regions, the average was less than 0.97 cases of LB per year; the lowest average indicators were noted in Odesa region – 0.22 and Kherson region – 0.09 cases of LB per year, respectively. The structured and aggregated data on average numbers of LB cases in dogs per year are shown in Table 2.

Table 2
Average number of cases of Lyme borreliosis in dogs per year by regions of Ukraine (x ± SD)

Region of Ukraine	Average number of cases of LB in dogs per year in the region
Chernihiv	1.57 ± 0.28
Kyiv	1.45 ± 0.86
city of Kyiv	1.36 ± 0.74
Cherkasy	1.35 ± 0.56
Sumy	1.32 ± 0.95
Khmelnytsky	1.22 ± 0.56
Vinnytsia	1.13 ± 0.34
Kharkiv	1.11 ± 0.73
Lviv	0.99 ± 0.52
Ternopil	0.97 ± 0.35
Zhytomyr	0.85 ± 0.48
Kirovograd	0.84 ± 0.29
Rivne	0.76 ± 0.33
Dnipropetrovs'k	0.75 ± 0.25
Ivano-Frankivsk	0.69 ± 0.53
Poltava	0.67 ± 0.25
Chernivtsi	0.66 ± 0.18
Zakarpattia	0.53 ± 0.27
Mykolaiv	0.35 ± 0.21
Zaporizhzhya	0.32 ± 0.15
Volyn	0.31 ± 0.15
Odesa	0.22 ± 0.08
Kherson	0.09 ± 0.05

Note: x – mean value, SD – standard deviation.

The highest average number of LB cases in dogs per year was observed in the northern regions: Chernihiv, Kyiv, Sumy regions and in the central districts of Cherkasy region (1.32–1.57 cases of LB per year), in the west – in Khmelnytsky, Vinnytsia,Kharkiv Lviv, Ternopil regions (0.97–1.22 cases of LB per year. In all the other regions of Ukraine, the average number of cases of LB in dogs per year ranged 0.85 to 0.09. The average number of cases of dog rabies per year decreased in the direction from the north-western territories to the south of Ukraine. The lowest average number of cases per year was observed in the south in Mykolaiv, Zaporizhzhya, Odesa and Kherson regions (0.09–0.35 cases per year) and in the westernmost part of Ukraine, in Zakarpattia (0.53 cases per year) and Volyn regions (0.31 cases per year, Fig. 1).

Dependence of the prevalence of LB in dogs on natural zones of Ukraine. Geo-coordinated indicators of the average annual number of LB cases in dogs are plotted on a map of Ukraine indicating the natural zones (Fig. 2). The highest average numbers of LB cases in dogs per year accounted for 1.22 in the forest-steppe zone and 1.02 LB cases per year in the broad-leaved forest zone, respectively. Further, in each the zone of the Ukrainian Carpathians and in the zone of mixed forests, the average indicators equaled 0.53 cases of LB per year, and the lowest average indicator was 0.41 cases of LB per year in the steppe zone. There is no data for the territory of the Crimean Mountains zone.

We compared groups of data on the average number of LB cases in dogs per year, divided according to the natural zones of Ukraine. As a result, a statistically significant difference ($P < 0.001$) was determined in the dependent variable between all the compared data groups (Table 3). No statistically significant difference was found when comparing groups of data with medians with close values that belonged to the zone of mixed forests and the zone of the Ukrainian Carpathians ($P = 0.265$). An insignificant difference in the dependent variable ($P = 0.052$) was found when comparing groups of data on the average number of LB cases in dogs per year belonging to the forest-steppe and the broad-leaved forest zones.

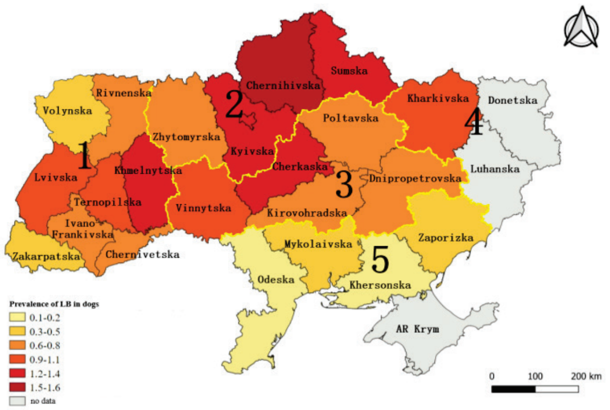

Fig. 1. Cartographic analysis of the prevalence of Lyme borreliosis in dogs across the regions of Ukraine: geographical division of the territory of Ukraine into regions: *1* – Western Ukraine: Volyn, Rivne, Lviv, Ternopil, Zakarpattia, Ivano-Frankivsk, Chernivtsi regions; *2* – Northern Ukraine: Zhytomyr, Kyiv, Chernihiv, Sumy regions; *3* – Central Ukraine: Vinnytsia, Cherkasy, Poltava, Kirovohrad, Dnipropetrovsk regions; *4* – Eastern Ukraine: Kharkiv, Luhansk, Donetsk regions; *5* – Southern Ukraine: Odesa, Mykolaiv, Kherson, Zaporizhzhya, Autonomous Republic of Crimea

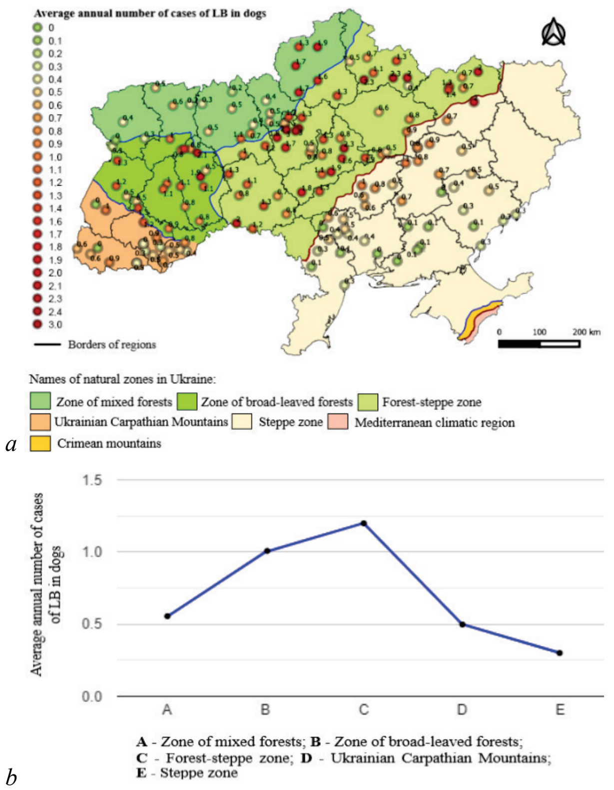

Fig. 2. Cartographic analysis of the prevalence of Lyme borreliosis in dogs depending on the natural zones of Ukraine (*a*), graph of average annual cases of LB in dogs by natural zones (*b*)

The territory of Ukraine is conventionally divided into seven zones of gross moisture (in mm): below 400; from 400 to 450; 450 to 500; 500 to 550; 550 to 600; 600 to 650 and above 600. In the zones with different indicators of the gross moisture, the indicators of the average number of cases of LB in dogs per year differed. The highest average value of 1.15 cases of LB per year was observed in the zone with the gross moisture of 550–600 mm, slightly lower indicators in zones with the gross moisture of 600–650 mm – 1.03 case of LB per year, in the zone with the gross moisture of 500–550 mm – 0.95 LB cases per year and in the zone with the gross moisture above 650 mm – 0.93 LB cases per year, respectively. The lowest average rate of 0.11 LB cases per year was noted in zones with the gross moisture below 400 and 400–450 mm, respectively (Fig. 3).

Table 3
Statistical analysis of the prevalence of Lyme borreliosis in dogs depending on the natural zones in Ukraine

Statistical indicators	Zone of mixed forests	Zone of broadleaved forests	Forest-steppe zone	Zone of the Ukrainian Carpathians	Steppe zone	Crimean mountains
n	23	21	44	18	42	–
Min–max	0.00–1.92	0.00–2.14	0.14–3.00	0.00–1.22	0.00–1.01	–
Median	0.53	1.02	1.22	0.53	0.41	–

Note: n – sample size of geo-coordinated indicators of the average number of cases of LB in dogs per year; min – minimum value; max – maximum value; Kruskal-Wallis H test – P < 0.001; there is a statistically significant difference in the dependent variable between all compared groups.

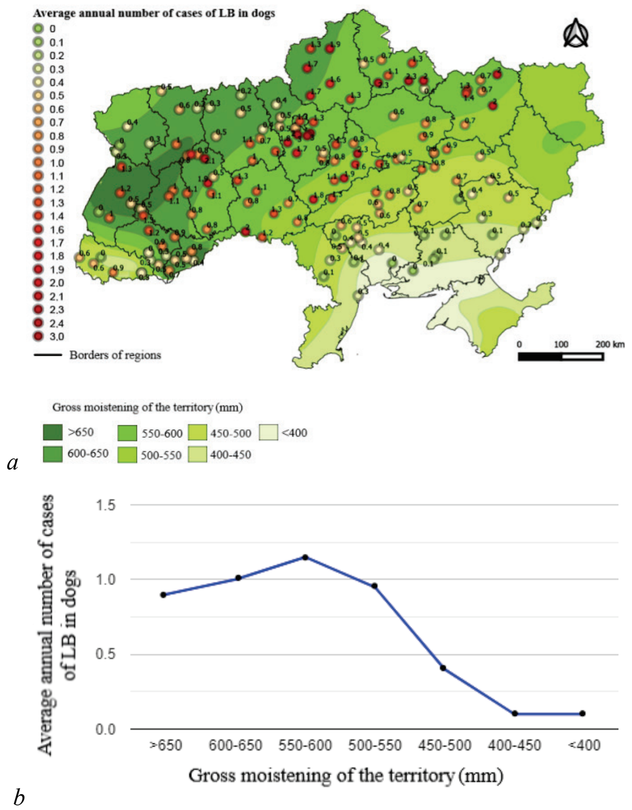

Fig. 3. Cartographic analysis of the prevalence of Lyme borreliosis in dogs depending on indicators of gross moisture in the territory of Ukraine (*a*), graph of the average annual incidence of dogs with Lyme borreliosis by gross moisture in the territory (*b*)

Comparison of the data groups regarding average number of cases of LB in dogs per year showed a statistically significant difference between them (P < 0.001), indicating the influence of the gross moisture of the territory on the prevalence of LB in dogs (Table 4). We found no statistically significant difference (P = 0.316) between the data groups of average indicators of the number of LB cases in dogs per year and the medians with similar values, which belonged to the zones with gross moisture of less than 400 and 400–450 mm. Also, no statistically significant difference (P = 0.118) was found when comparing the groups of data regarding the zones with the gross moisture of 500–550, 550–600 and 600–650 mm.

According to the average annual air temperature, the territory of Ukraine is conventionally divided into 11 zones: below 3 °C, from 3 to 4 °C, 4 to 5 °C, 5 to 6 °C, 6 to 7 °C, 7 to 8 ° C, 8 to 9 °C, 10 to 11 °C, 11 to 12 °C and above 12 °C. We received data from the areas where the annual air temperature ranges 6–7 °C to 9–10 °C. No data were received from other areas where the average annual temperature ranges <3 °C to 5–6 °C, since those areas are located in the territory of the mountain ranges of the Ukrainian Carpathians. There are also no data from the territories where the annual average air temperature is 11–12 °C to >12 °C; those territories are located on the Crimean Peninsula, occupied by the Russian Federation. The data from the areas with 10–11 °C average annual air temperature were insufficient for statistical processing (n = 3) and thus, we were unable to characterize those areas regarding LB in dogs. Nonetheless, the zones smoothly transition into one another and having the indicators from the territories that are on the border of the 10–11 °C zone and the 9–10 °C zone, the data of the average number of LB cases in dogs per year were assigned to the zone with the annual average air temperature of 9–10 °C. Therefore, the highest indicators of the average number of LB cases in dogs per year – 0.92 – were observed in territories with the average annual air temperature of 7–8 °C. In territories with the average annual air temperature of 6–7 °C, there occurred 0.83 cases of LB per year. Further, in the zone with with the average annual air temperature of 8–9 °C, the indicator was significantly lower – 0.54 LB cases per year. The lowest average number of LB cases in dogs per year – 0.12 – was observed in the areas with the average annual air temperature of 9–10 °C (Fig. 4).

Table 4
Statistical analysis of the prevalence of Lyme borreliosis in dogs depending on gross moisture

Statistical indicators	Gross moisture of the territory, mm						
	<400	400–450	450–500	500–550	550–600	600–650	>650
n	12	16	55	52	71	60	19
Min–max	0.00–0.30	0.14–0.40	0.00–1.03	0.52–2.3	0.00–3.00	0.00–2.14	0.51–1.32
Median	0.10	0.11	0.42	0.95	1.15	1.03	0.93

Note: n – sample size of geo-coordinated indicators of the average number of cases of LB in dogs per year; max – maximum value; Kruskal-Wallis H test – P < 0.001 – there is a statistically significant difference in the dependent variable between all the groups.

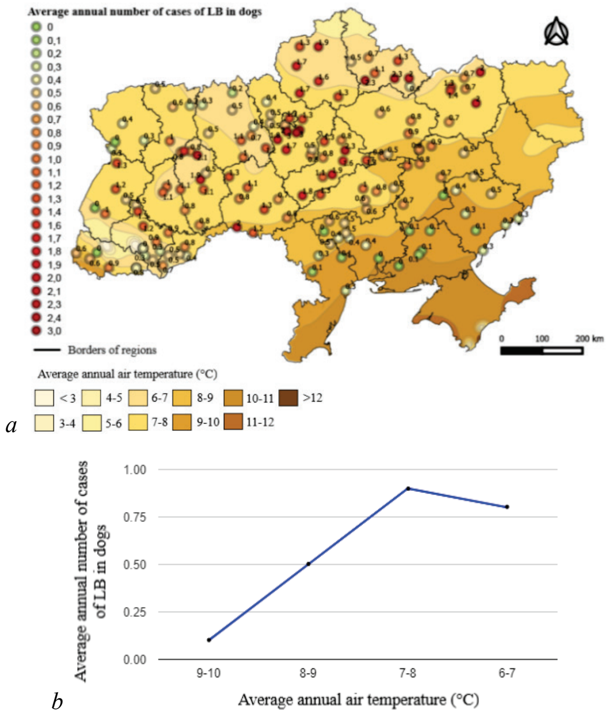

Fig. 4. Cartographic analysis of the prevalence of Lyme borreliosis in dogs depending on the indicators of the average annual air temperature (*a*), graph of the average annual incidence of dogs with Lyme borreliosis depending on the average annual air temperature (*b*)

When comparing the data groups of the average number of LB cases in dogs per year, which belonged to the 6–7, 7–8, 8–9 and 9–10 °C zones, a statistically significant difference was determined (P < 0.001), indicating a relationship between the prevalence of LB in dogs and air temperature (Table 5). It should be noted that the comparison of the data groups of average indicators of the number of LB cases in dogs per year with medians close in value, which belonged to 6–7 and 7–8 °C average annual air temperature zones, indicated an insignificant difference in the dependent variable of those groups (P = 0.057). This indicates that the prevalence of LB in dogs in territories with 6 to 8 °C average annual air temperature has no significant difference.

Prevalence of LB in dogs depending on the average temperature of the soil surface in the warm season – in July. According to the average soil temperature in July, the territory of Ukraine is conditionally divided into 9 zones: below 14, 14–16, 16–18, 18–20, 20–22, 22–24, 24–26, 26–28, and above 28 °C. Therefore, the highest average number of LB cases in dogs per year, 1.22, was observed in the zone with the average soil surface temperature in July measuring 22 to 24 °C. Further, the average rate of 0.83 LB cases per year was seen in areas with the average soil surface temperature of 24–26 °C and 0.65 cases of LB in dogs per year in the zone with 20–22 °C soil surface temperature. The lowest average number of LB cases in dogs per year – 0.14 – was noted in the territories with relatively high average summer soil surface temperatures, accounting for more than 28 °C. We received no data from the territories of the Ukrainian Carpathians, where the average soil surface temperatures in July range <14 to 16–18 °C (Fig. 5).

Table 5
Statistical analysis of the prevalence of Lyme borreliosis in dogs depending on the average annual air temperature

Statistical indicators	Average air temperature per year, °C										
	< 3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	>12
n	–	–	–	–	78	95	68	44	–	–	–
Min–max	–	–	–	–	0.00–2.31	0.00–3.00	0.00–2.02	0.10–0.41	–	–	–
Median	–	–	–	–	0.83	0.92	0.54	0.12	–	–	–

Note: n – sample size of geo-coordinated indicators of the average number of cases of LB in dogs per year; min – minimum value; max – maximum value; Kruskal-Wallis H test – P < 0.001 – there is a significant difference in the dependent variable between the groups of data belonging to the territories with the average annual air temperature of 6–7, 7–8, 8–9 and 9–10 °C.

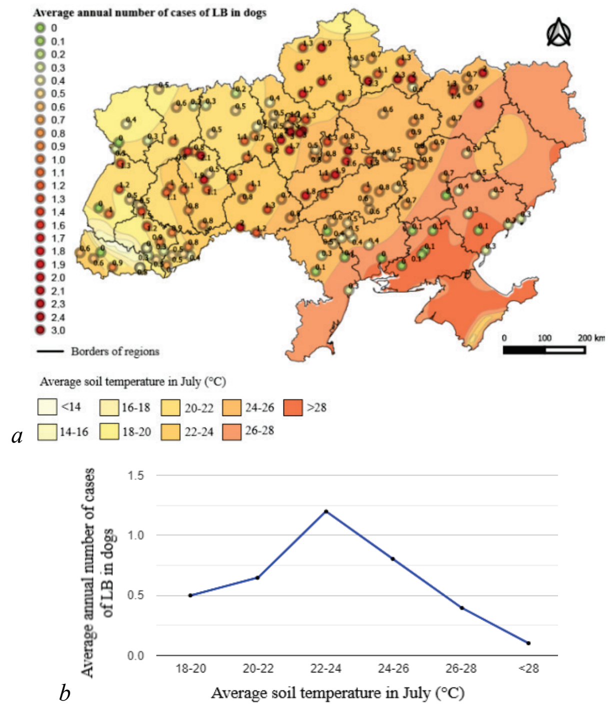

Fig. 5. Cartographic analysis of the prevalence of Lyme borreliosis in dogs depending on average temperature of the soil surface (*a*), graph of the average annual incidence of dogs with Lyme borreliosis depending on average soil surface temperature (*b*)

A between-group comparison of the mean number of LB cases in dogs per year, divided by mean soil temperature, indicated a statistically significant difference between the groups (p<0.001). This suggests that soil surface temperature has an effect on the prevalence of LB in dogs (Table 6).When comparing the data groups with medians close in value, which belonged to the zone 20–22 °C average summer soil surface temperature and the zone with 18–20 °C average summer soil surface temperature, a slight difference was determined in the dependent variable of the data groups (p=0.053). No statistically significant difference (p=0.165) was found between the data group that belonged to the zone with 20–22 °C summer soil surface temperature and the data group that belonged to the zone with 24–26 °C average summer soil surface temperature.

Table 6
Statistical analysis of the prevalence of Lyme borreliosis in dogs depending on average summer soil surface temperature

Statistical indicators	Average temperature of soil surface (°C)								
	<14	14–16	16–18	18–20	20–22	22–24	24–26	26–28	>28
n	–	–	–	31	57	61	71	45	20
Min–max	–	–	–	0.00–1.31	0.20–2.11	0.42–3.00	0.00–2.32	0.00–0.72	0.00–0.32
Median	–	–	–	0.54	0.65	1.22	0.83	0.40	0.14

Note: n – sample size of geo-coordinated indicators of the average number of cases of LB in dogs per year; min – minimum value; max – maximum value; Kruskal-Wallis H test – P < 0.001 – there is a significant difference in the dependent variable between the groups of data belonging to areas with the average soil surface temperature of 18–20, 20–22, 22–24, 24–26, 26–28 and >28 °C.

The analysis of the respondents' messages regarding the methods used to diagnose LB in dogs showed that to confirm this diagnosis, most respondents (86 out of 274; 31.4%) reported the use of immunochromatographic analysis, a lower number of the respondents used enzyme immunoassay methods for diagnosis analysis and immunochromatographic analysis (62 out of 274; 22.6%). Only 17.9% of the respondents (49 out of 274) diagnosed LB using the enzyme immunoassay methods. Also, 1.5% of doctors (4 out of 274) used Western-blot for diagnosis. In general, the majority of veterinarians (201 out of 274; 73.4%) confirm the diagnosis based on the detection of antibodies against Bb in the animal's plasma or blood serum. Lyme disease has been diagnosed using polymerase chain reaction by 10.6% of the respondents (29 out of 274). As with combined molecular diagnostic and serological methods, 8.0% of the veterinarians (22 out of 274) used polymerase chain reaction and the enzyme immunoassay methods for diagnosis, and another 7.3% of the respondents additionally used immunochromatographic analysis (20 out of 274). Some respondents (2 out of 274; 0.7%) indicated that they had diagnosed Lyme disease only based on the clinical manifestations: arthritis, neurological disorders, erythema at the site of a tick bite, lameness, fever, and enlarged lymph nodes.

According to groups of clinical symptom complexes of LB manifestation in dogs, over a third of the respondents (102 out of 274; 37.2%) observed Lyme arthritis alone. A total of 16.1% (44 out of 274) of the veterinarians reported Lyme arthritis and neuroborreliosis in dogs; 14.6% (40 out of 274) – Lyme arthritis and Lyme nephritis; 9.1% (25 of 274) – Lyme arthritis, Lyme nephritis and neuroborreliosis; 7.7% (21 of 274) – Lyme arthritis, Lyme nephritis and erythema; 6.2% (17 out of 274) – Lyme arthritis and erythema; Lyme arthritis, 0.7% (2 of 274) – Lyme nephritis and Lyme carditis. A total of 5.5% (15 out of 274) of the respondents reported encountering only symptoms of Lyme nephritis, 1.5% (4 out of 274) noted only erythema without other manifestations, and 0.7% observed the dogs to have only clinical symptoms of Lyme carditis (2 of 274) or neuroborreliosis (2 of 274). Generalized information reported by respondents about the clinical symptom complexes of LB in dogs is shown in Table 7.

In general, veterinarians most often reported symptoms of Lyme arthritis (251 out of 274; 91.6%) and Lyme nephritis (103 out of 274; 37.6%). A third of the respondents (92 out of 274; 33.6%) noted manifestations of Lyme disease in the form of neuroborreliosis; 33.6% (92 out of 274), 15.3% of respondents (42 out of 274) noted characteristic erythema at the site of a tick bite, and the fewest doctors reported Lyme symptoms carditis (4 out of 274; 1.5%). Generalized information about the frequency of the manifestations of individual LB symptom complexes in dogs according to the respondents is given in Table 8.

For the treatment of LB in dogs, the respondents most often used beta-lactam antibiotics. Tetracyclines were used by 79.1% of the respondents (219 out of 274), 18.4% of the respondents (51 out of 274) used cephalosporins, and 10.5% of the respondents (29 out of 274) used penicillins. Macrolide antibiotics were used by 14.4% of the doctors (40 out of 274); aminoglycoside antibiotics – by 7.9% of the respondents (22 out of 274); a group of carbapenems – by 2.5% of the respondents (7 out of 274) and one respondent reported the use of antibiotics from the group of lincosamides (1 out of 274; 0.4%). Generalized information on the use of different groups of antimicrobial drugs by the veterinarians for the treatment of LB is given in Table 9.

Table 7
Analysis of veterinarians' reports on clinical manifestations of Lyme borreliosis in dogs by groups of symptom complexes (n = 274)

Name of clinical symptom complexes	Number of reports of individual symptom complexes	Percentage of reports of individual symptom complexes
Lyme arthritis	102	37.2
Lyme arthritis / Neuroborreliosis	44	16.1
Lyme arthritis / Lyme nephritis	40	14.6
Lyme arthritis / Lyme nephritis / Neuroborreliosis	25	9.1
Lyme arthritis / Lyme nephritis / Erythema	21	7.7
Lyme arthritis / Erythema	17	6.2
Lyme nephritis	15	5.5
Erythema	4	1.5
Lyme arthritis / Lyme nephritis / Lime carditis	2	0.7
Lime carditis	2	0.7
Neuroborreliosis	2	0.7
Total	274	100.0

Note: n – total number of veterinarians' reports of clinical manifestations of LB in dogs.

Table 8
Analysis of the frequency of the manifestations of individual symptom complexes of Lyme borreliosis in dogs (n = 274)

Name of clinical symptom complexes	Frequency of mentioning of individual symptom complexes by veterinarians	Share of mentions of individual symptom complexes by veterinarians
Lyme arthritis	251	91.6
Lyme nephritis	103	37.6
Neuroborreliosis	92	33.6
Erythema	42	15.3
Lyme carditis	4	1.5

Note: see Table 7.

Table 9
Analysis of the use of different groups of antibiotics for the treatment of Lyme borreliosis by veterinarians of Ukraine (n = 274)

Names of antibiotic groups	Frequency of use of different groups of antibiotics for the treatment of LB in dogs	Percentage of the use of different groups of antibiotics for the treatment of LB in dogs
Tetracyclines	219	79.9
Cephalosporins	51	18.6
Macrolides	40	14.6
Penicillins	29	10.6
Aminoglycosides	22	8.0
Carbapenems	7	2.6
Lincosamides	1	0.4

Note: see Table 7.

Almost half of the respondents (137 of 285; 48%) reported seeing an increase in the incidence of LB in dogs. Thus, most answers confirming the increase in the LB incidence in dogs were received from the city of Kyiv, where 29 out of 31 respondents reported an increase in the frequency of LB cases in dogs, from Cherkasy (14 out of 17), Lviv region (9 out of 11), Khmelnytskyi (7 out of 10), Kyiv region (16 out of 24), Ternopil region (6 out of 9), Chernihiv region (6 out of 10), Sumy region (7 out of 12), Poltava region (4 out of 7) and Vinnytsia region (5 out of 9), respectively. In other regions of Ukraine, the share of respondents who reported an increase in the frequency of LB cases in dogs was 50% or less. Generalized information about the respondents' observations regarding the dynamics of the frequency of LB cases in dogs is presented in Table 10.

Table 10
Analysis of the observation of veterinarians on the frequency of cases of dogs suffering Lyme borreliosis in the regions of Ukraine

Region of Ukraine	Total number of surveyed veterinarians in the region	Number of veterinarians who observe an increase in the incidence of LB in the region	Share of veterinarians who observe an increase in the incidence of LB in the region
city of Kyiv	31	29	94
Cherkasy	17	14	82
Lviv	11	9	82
Khmelnytsky	10	7	70
Kyiv	24	16	67
Ternopil	9	6	67
Chernihiv	10	6	60
Sumy	12	7	58
Poltava	7	4	57
Vinnytsia	9	5	56
Kharkiv	16	8	50
Zhytomyr	16	7	44
Kirovograd	9	4	44
Dnipropetrovsk	14	4	44
Ivano-Frankivsk	13	5	38
Rivne	8	3	38
Transcarpathian	8	2	25
Chernivtsi	9	2	22
Volyn	9	0	0
Zaporizhzhya	11	0	0
Mykolaiv	12	0	0
Odesa	9	0	0
Kherson	11	0	0
Total	285	137	48

Discussion

Our study showed that veterinarians are diagnosing LB in dogs throughout Ukraine, but there is a difference in prevalence in different geographic areas. The highest average annual number of diagnosed cases of LB in dogs (0.97 cases per year or more) was observed in the northern regions of Ukraine: Chernihiv, Sumy, Kyiv regions, and the city of Kyiv; in the central regions: Cherkasy and Vinnytsia regions; in the west: Khmelnytskyi, Ternopil, Lviv regions and in the east in the Kharkiv region. Much fewer cases were recorded in the southern regions, in the region of the Carpathian Mountains, and the northernmost territories of Ukraine, measuring 0.09 to 0.53 cases of LB in dogs per year. Cartographic analysis of the prevalence of LB in dogs across the regions of Ukraine indicated similar results regarding the general trends in the prevalence and incidence of LB in people in Ukraine for 20 years (2000–2019), reporting significantly higher annual incidence of LB in people in northern, in the central and western regions of Ukraine compared with the southern territories (Rogovskyy et al., 2020).

According to the types of landscapes, the territory of Ukraine is divided into seven natural zones: the zone of mixed forests, the zone of broad-leaved forests, the forest-steppe zone, the Ukrainian Carpathians, the steppe zone, and the Crimean Mountains. Each of the zones has its own distinctive characteristics in terms of geology, topography, climate, hydrology, soils, vegetation, and animal life. Generalized information on the climatic characteristics of individual natural zones, which, in our opinion, can affect the prevalence of LB, is provided in Table 11.

The prevalence of LB in dogs varies depending on the natural zones. The comparison of the groups of data on average annual number of LB cases in dogs divided according to the natural zones revealed a significant difference between all the compared groups ($P < 0.001$). This indicates that climatic conditions and other natural factors characteristic of the natural zones of Ukraine have an indirect effect on the incidence of Lyme disease in dogs.

The highest rates of the average number of cases of LB in dogs per year were observed in the forest-steppe zone and the zone of broad-leaved forests, accounting for 1.22 and 1.02 cases, respectively. There is no statistical difference between the dependent variables of those zones ($P = 0.052$). It is likely that the higher rates of LB in dogs in those natural areas are related to climatic, natural factors that favour the circulation of *Borrelia* infection in ticks, reservoir animals, and domestic dogs. The forest-steppe zone and the zone of broad-leaved forests run through the eastern, central, and western territories of Ukraine. Those zones border each other and are characterized by a sufficiently warm average daily temperature in the warm season (17–24 °C) and a high moisture coefficient (1.2–2.8), which probably also has a favourable effect on the spread of borreliosis infection across all links of the epizootic chain. The forest-steppe zone accounts for about 34.0% of the territory of Ukraine. This zone includes the administrative territories of Vinnytsia, Cherkasy regions, partly the southeastern territories of Zhytomyr, Kyiv, and Chernihiv regions, most of the northwestern territory of Sumy region, and partly, the northern territories of Kirovohrad and Kharkiv regions. Northern forest-steppe landscapes are sufficiently moistened, and southern forest-steppe landscapes have developed in the conditions of a more arid climate, which also has an indirect effect on the frequency of LB cases in dogs.

Table 11
Characteristics of natural zones of Ukraine by climatic indicators and prevalence of LB in dogs

Climate indicators	Forest-steppe zone	Zone of broad-leaved forests	Mixed forest zone	Ukrainian Carpathian Mountains	Steppe zone	Crimean mountains
Average daily temperature in July	Changes from northwest to south from 18.0 to 22.0 °C	17–24 °C	Varies from north to south from 17.0 to 19.5 °C	16–18 °C. On mountain peaks 7–8 °C	Varies from north to south from 20.0 to 24.0 °C	22–24 °C
Coefficient dampening	In the west up to 2.8. In the south it decreases to 1.2–1.4	2.6–2.8	Varies from north to south from 2.8 to 1.9	2.0 and more	Varies from north to south from 1.2 to 0.8	0.3–1.0
Prevalence of LB in dogs	1.2	1.0	0.5	0.5	0.3	–

Cartographic analysis visually indicates that the average number of LB cases in dogs per year decreases in the southern direction, where the landscapes of the forest-steppe zone become more arid. The vegetation cover of the forest-steppe zone is represented by remnants of steppe meadows, steppe, and forest types of vegetation. In turn, the zone of broad-leaved forests is located in the western part of Ukraine between the zone of mixed forests, the forest-steppe zone, and the Ukrainian Carpathians. The basis for the selection of this zone was the presence of broad-leaved landscapes that account for almost 7.0% of the territory of Ukraine. The zone of broad-leaved forests partially covers the northern territories of Lviv and Ivano-Frankivsk regions, the southern regions of Volyn and Rivne regions, and extends over most of the territories of Ternopil and Khmelnytskyi regions. The zone of mixed forests is characterized by a moderately warm climate where the ratio of heat and moisture is close to optimal. Hornbeam thickets and beech forests predominate in thas zone.

In the natural zone of mixed forests, the average number of cases of LB in dogs was 0.53 cases per year, which is two times lower than in the zone of broad-leaved forests and 2.4 times less than in the forest-steppe zone. The zone of mixed forests is located in the north of Ukraine and occupies about 20.0% of the territory of the country. Most of the northern districts of Volyn, Rivne, Zhytomyr, Kyiv, Chernihiv, and Sumy regions are located in this natural zone. The zone of mixed forests is characterized by a moderate continental climate and a positive moisture balance, which contributes to the presence of a dense hydrographic network. Most natural territories are coniferous and broad-leaved forests, meadows, and swamps. The main types of vegetation in the zone of mixed forests are forest, meadow, and swamp vegetation. Forests occupy about 30% of the zone, mainly pine forests – 60%, and the other 40% – pine-oak, pine-oak-hornbeam, oak-hornbeam, and alder forests. Meadow areas make up about 10% of the territory of the mixed forest zone. More dry territories are located mainly in the places of cut-down forests. In lowland and transitional wetland areas, the vegetation is represented by grass and grass-moss associations. Despite the fact that the natural zone of mixed forests has all the conditions for maintaining the circulation of *B. burgdorferi*, the average number of cases of LB in dogs per year was lower than in the zone of broad-leaved forests and the forest-steppe zone. We assume that the lower incidence of LB dogs in the zone of mixed forests is due to the fact that the northern regions have a low population density (http://pop-stat.mashke.org/ukraine-division.htm) and large forested areas, high waterlogging and low-fertility soils. Those regions include the northern regions of Volyn, Rivne, Zhytomyr, Kyiv, Chernihiv, and Sumy regions. Although, according to the veterinarians, the incidence in dogs in Chernihiv region was the highest in Ukraine, amounting to 1.57 cases of LB per year, slightly lower numbers of cases of LB were observed in Kyiv region – 1.45 and in Sumy region – 1.32 cases per year, respectively. Also, it should be noted that part of Kyiv and Zhytomyr regions are located in the Chornobyl exclusion zone, which is about 2,600 km2 of the territory of Ukraine. However, this does not mean that in the natural ecosystems of sparsely populated areas and the Chornobyl exclusion zone, the LB pathogen is less common than in other areas. For example, this is indicated by studies of ixodid ticks from the Chornobyl exclusion zone, where 13.5% of the studied *I. ricinus* were carriers of *B. burgdorferi s.l.*, which is higher than for *I. ricinus* studied at the same time in the city of Kyiv – 10.4% (Rogovskyy et al., 2019). This provides the basis for further study of the circulation of *B. burgdorferi s.l.* in natural ecosystems and predicttng the risks of the spread of the borreliosis pathogen in an urbanized environment.

In the Ukrainian Carpathians, the average annual number of cases of LB in dogs is 0.53 cases per year. The Carpathian region of climatic zoning is a taxonomic unit of agro-climatic zoning of Ukraine. The territories of the Carpathian Mountains include the administrative territories of Zakarpattia region and the southern parts of Lviv and Ivano-Frankivsk regions. The Ukrainian Carpathians are formed by a stratified system: high-mountain, medium-mountain, low-mountain, and foothill strata. At the foot of the mountains, the absolute height of the Carpathian mountain system is 120–140 m, in the intermountain areas 500–800 m, along the mountain ridges 1,500–2,000 m, and the highest peak – Hoverla Mountain – 2,061 m. The climate is warm, and moderately continental. In the foothills and on the slopes of the mountains, there are deciduous forests, represented mainly by oaks, to a lesser extent by hornbeams and beeches. Mixed beech-coniferous forests begin in the high areas, becoming coniferous higher up, with the soil covered with moss, and the meadows covered with shrubs. At the highest points, there are wastelands and subalpine meadows. We assume that the incidence of LB in domestic dogs in the natural zone of the Ukrainian Carpathian Mountains is lower, compared with the other natural zones, due to the fact that the altitude above sea level has an effect on the survival and distribution of the ranges of vectors of *B. burgdorferi s.l.* – ixodid ticks. Studies indicate that there is a clear biological relationship between increasing altitude and decreasing *I. ricinus* abundance at 620–1,270 m above sea level, and at higher altitudes, there are fewer available host animals for ixodid ticks (Randolph, 2004; Danielová et al., 2006; Materna et al., 2008). This suggests that in the intermountain areas of the Carpathian Mountains, where the height above sea level is 500–800 m, the risk of borreliosis infection in animals and humans is lower. Also, in the zone of mixed forests in the high-altitude areas of the Carpathian Mountains, the natural conditions determine the low population density (http://pop-stat.mashke.org/ukraine-division.htm), and probably therefore the incidence of the causative agent of LB among the population and companion animals, as seen in feedback from the respondents.

The lowest incidence of LB in dogs, 0.41 cases per year, was observed in the steppe natural zone, which is characterized by steppe plain landscapes and spans from the southwest to the northeast in the southern part of Ukraine. The territory of this zone accounts for about 40% of the territory of Ukraine. The steppe zone includes the southern regions of Sumy, Poltava, and Kirovohrad regions, most of Odesa region, almost the entire territory of Mykolaiv region, and the administrative territories of Dnipropetrovsk, Zaporizhzhya, Kherson, Luhansk, and Donetsk regions and Crimea. In the steppe zone, the climate is hot and moisture is insufficient. Grassy steppe vegetation predominates, but it is replaced by agricultural crops. In the south, steppe cereal crops predominate: *Stipa* spp., *Festuca* spp., *Koeleria* spp., etc. In the north, the vegetation is represented by meadow-steppe forbs, as well as floodplain forests that occur in river valleys. It is known that *I. ricinus* and *D. reticulatus* are less common species of ixodid ticks in the steppe areas of the landscape. In the past, studies in the territories of Odesa, Mykolaiv, Dnipropetrovsk, Kherson, Zaporizhzhya, Donetsk, Luhansk and AR Crimea seeking for the southern border of the range of *I. ricinus* indicate that ticks of this species are found in all southern territories with different degrees of population density and percentage of dominance, compared with the other types of hard ticks. Basically, in the steppe zone, *I. ricinus* is more often confined to forests, forest strips, wet places with developed herbaceous vegetation, it is also found in parks and green areas around cities. However, *I. ricinus* has not been recorded in areas with typical steppe landscapes, which occupy the main area of the natural steppe zone (Akymov et al., 1996). In the studies conducted to determine the epidemiological significance of ixodid ticks in the recreational zones of the northwestern Azov region of Zaporizhzhya region, it was indicated that the range and number of *I. ricinus* increases under anthropogenic conditions in the direction of more southern territories. The same study reported that in 2009, the causative agent of borreliosis was detected in 13.3% of the examined *I. ricinus*, which was comparatively more than in the studies conducted in 2006 – 8.7%, respectively. Antibodies to *B. burgdorferi s.l.* were also detected in 25.7% of blood serum tested using the ELISA method when a diagnosis of LB was suspected in humans (Voronova et al., 2009). An equally important vector of the causative agent of borreliosis – *D. reticulatus* ticks – was found in wet biotopes near water bodies and in urbanized biocenoses in the territories of the natural steppe zone of southern Ukraine, in Odesa, Mykolaiv, Kherson, Zaporizhzhya, Donetsk regions (Fedonyuk et al., 2020). In the natural zone of steppe in arid regions, the population density is relatively low in Kherson region and in some parts of Zaporizhia, Mykolaiv, Odesa, and Kirovohrad regions, which may also had an effect on the respondents' answers and the spread of LB among domestic animals in these regions (http://pop-stat.mashke.org/ukraine-division.htm).

Our study determined the impact of individual climate indicators on the prevalence of canine borreliosis in Ukraine. Such indicators include gross moisture of the territory, average annual air temperature, and soil surface temperature. Therefore, in areas with gross moisture values ranging 500 to >650 mm, the incidence of Lyme borreliosis in dogs was relatively higher – 0.93–1.15 cases of LB per year – than in areas with gross moisture values of 500 mm and fewer, 0.10–0.42, cases of LB per year ($P < 0.001$). With a decrease in gross moisture indicators of territories from 500 mm and below, a sharp decrease in the incidence of Lyme borreliosis in dogs was determined. Such territories include the southern regions of Ukraine and the Carpathian mountain ranges. It is known that Ixodes ticks need the stability of the internal water balance to survive. Usually, the comfort threshold of relative humidity of the environment for those ticks ranges from 86–96%. While searching for a host, ticks lose a significant part of their body moisture, and therefore, to restore their water balance, they need to return to a moist environment – the litter layer (Herrmann et al., 2015). The biological need of hard-bodied ticks to maintain the body water balance can explain the decrease in their number and activity in more arid regions, and, as a result, affect the decrease in the intensity of circulation of *B. burgdorferi s.l.* (in our case, these are the southern territories of Ukraine). Previous studies indicate that temperature and humidity are among the main factors affecting the activity, survival, and maintenance of tick populations, presence of host animals, their survival, and the transmission of pathogens of transmissible diseases (Dumic et al., 2018).

The analysis of the influence of the average annual air temperature on the prevalence of LB in dogs does not indicate that Lyme disease in dogs occurred more often in the northern, central, and eastern regions, where the average annual air temperature in the range from 6 to 8 °C was recorded. In those regions, the incidence accounted for 0.83–0.92 cases per year. When the average annual air temperature increased from 8 to 10 °C in the southern regions of Ukraine, the incidence of Lyme borreliosis in dogs decreased from 0.54 to 0.12 cases per year from north to south. In the USA, studies of the impact of the climate change on the incidence of Lyme disease in the population showed similar results to ours, which indicate significantly higher incidence rates in 7–9 °C range of average annual temperatures (Dumic et al., 2018). Scientists indicate that the changes in temperature fluctuations affect the number of ixodid ticks and the diseases they carry. This may depend on the general temperature over the year and could vary across areas. The relationship between temperature fluctuations and the dynamics of tick-borne diseases is a complex process, because the spread of pathogens depends on the timing of tick feeding at different stages of the life cycle and the availability of host animals (Hancock et al., 2011; Herrmann et al., 2013).

Also, our study demonstrates the relationship between the average annual number of LB cases in dogs and soil temperature ($P < 0.001$). The highest numbers of LB cases in dogs were recorded in territories with 22–24 °C soil surface summer temperature, equaling the average of 1.22 cases per year, and in territories with 24–26 °C soil surface temperature – 0.83 cases, respectively ($P = 0.165$). Regions with summer temperature indicators of the soil surface in the range from 22 to 26 °C are located in the northern and central regions of Ukraine. When the surface temperature of the soil increased above 26 °C in the southern regions of Ukraine, the incidence of LB in dogs decreased from north to south from 0.40 to 0.14 cases per year. In the western regions of Ukraine, where the summer soil surface temperatures ranges 18–20 to 20–22 °C, the average annual incidence of Lyme disease in dogs was approximately twice as low, accounting for 0.54 and 0.65 cases, respectively ($P = 0.053$). The surface temperature of the soil is an equally important factor influencing the spread of ixodid ticks and the causative agent of Lyme borreliosis. Research conducted in the Czech Republic indicates that fluctuations in soil temperature amplitudes affect the number and activity of ixodid ticks. The results of the study indicated that *I. ricinus* is a "mesophilic" species of ixodid tick, and when the soil temperature increases during the day, they become more active in searching for a host (Hubálek et al., 2003).

All over the world, there is a trend towards an increase in the incidence of Lyme disease, which is associated with the climate change, increased anthropogenic impact on the natural environment, and other ecological and geographical factors (Hofhuis et al., 2006; Hubálek, 2009; Rizzoli et al., 2011; Strle et al., 2018; Nebogatkin et al., 2020). Close contact of humans and companion animals with the natural environment increases the risk of tick-borne infections. The influence of climatic factors on fluctuations in Lyme disease outbreaks has been described in many studies. Scientists report a number of climatic factors that affect the risk of occurrence, distribution, and endemicity of LB, such as temperature, humidity, altitude above sea level, duration of the growing season, and other abiotic and biotic environmental factors (Semenza et al., 2018; Li et al., 2019; Omazic et al., 2019; Bregnard et al., 2020). For example, vegetation and the presence of host animals influence the phenology and distribution of tick habitats and, as a result, the spread of the diseases they carry. Lyme disease is a classic example that demonstrates the connection between the natural environment and the spread of a pathogen among humans and domestic animals (Bouchard et al., 2019). In Ukraine, as in other countries, Lyme borreliosis has a heterogeneous distribution among the population, domestic animals, among populations of wild animals, and ixodid ticks. First of all, it is related to climatic factors that affect ecosystems, changing the distribution of fauna and flora, the redistribution of hard-bodied tick populations, and the spread of tick infections (Patz et al., 2005). If earlier outbreaks of Lyme borreliosis were observed in certain endemic regions, now there is an increase in the geographical scope of disease occurence (Capelli et al., 2012). In Ukraine and European countries, the main carriers of the borreliosis complex of spirochetes – *Borrelia burgdorferi sensu lato* – are considered to be two species of ticks, *I. ricinus* and *D. reticulatus* (Abdullah et al., 2018; Kubiak et al., 2022). In the territory of Ukraine, *Borrelia* infection in *I. ricinus* accounts for 68.8%, and 22.9% in *D. reticulatus* (Nebogatkin et al., 2020). In Ukraine, in 2000, Lyme disease was included in the list of infectious diseases of the population subject to records. As for cases of Lyme borreliosis in animals, unfortunately, the disease is not being officially recorded. Scientists also point out that dogs can be a kind of marker for displaying the prevalence of the borreliosis pathogen in the environment and used to predict and assess the risks of Lyme disease outbreaks in population (Hamer et al., 2009; Galluzzo et al., 2020).

Based on the results of the survey data analysis, we determined that in most cases, Ukrainian veterinarians preferred serological diagnosis of LB in dogs (73.4%). More often, veterinarians used the method of immunochromatographic analysis (31.4%). This is probably due to the speed of the analysis and the availability of diagnostic tests on the market in Ukraine. A total of 17.9% of veterinarians used the IFA diagnostic method. A small number of respondents indicated that they used immunoblotting for the qualitative detection of specific antibodies against *B. burgdorferi s.l.* to diagnose LB in dogs (1.5%). Molecular PCR diagnosis was used in 10.6% of the cases, and the combination of ELISA and PCR methods was used in 8.0% of the cases, respectively. Serological methods of diagnosis, such as ELISA and the molecular method of PCR diagnosis, require the availability of certain equipment and qualified personnel to conduct the tests. Usually, veterinary clinics do not have the opportunity to conduct ELISA and PCR on their own, so samples have to be sent to specialized diagnostic laboratories of veterinary medicine, which are located in large cities of regional importance, for example, Kyiv, Lviv, Kharkiv, Odesa, etc. This complicates the logistical process of transporting samples for diagnosis and takes more time. It also increases the cost of diagnosis, which may be the reason why veterinarians use those methods less often. In the USA, for example, veterinarians diagnose LB in dogs according to the following criteria: typical clinical signs; exclusion of diseases with similar symptoms (differential diagnosis); pronounced reaction to antibiotic treatment; clear contact with a tick or living in an endemic area; presence of antibodies in blood serum (Jacobson et al., 1996). In some sources, it is noted that serological diagnosis in dogs is the only recommended means of assessing exposure to *B. burgdorferi s.l.*, but the presence of antibodies against C6, VlsE, OspC (in non-vaccinated animals), OspF indicates only that a dog was in contact with the causative agent of borreliosis and it is not an evidence of the cause of the clinical signs or their development in the future (Littman et al., 2018). Some scientists indicate the expediency of using the PCR method to detect antigen DNA in dogs with clinical symptoms of borreliosis. Their data show that of 92 dogs with clinical signs of LB, 40.2% had antibodies to *B. burgdorferi s.l.* and 45.1% of the dogs had a developed immune reaction, the presence of *Borrelia* DNA was determined, thus PCR can contribute to improving the diagnosis of LB in dogs (Skotarczak et al., 2005). Data from the survey among respondents indicate the need for further study and development of a science-based algorithm with a comprehensive approach to the diagnosis of Lyme disease in dogs and further implementation in the clinical veterinary practice.

It is believed that the difference in the manifestations of the clinical symptom complexes of Lyme disease is related to the genetic heterogeneity of the *B. burgdorferi s.l.* complex (Pachner et al., 2004). For example, the genotype *B. burgdorferi sensu stricto* causes the development of Lyme arthritis, neurological symptoms of LB are associated with *B. garinii*, while *B. afzelii* is mainly associated with skin manifestations of Lyme disease in humans. *Borrelia burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii* are considered to be pathogenic *Borrelia* species for dogs (Hovius et al., 1999; Littman et al., 2018). For example, in the USA, only the genotype *B. burgdorferi sensu stricto* was detected in the dogs suffering LB. In Europe, dogs are mainly infected by *B. burgdorferi sensu stricto*, but also probably with *B. afzelii*, *B. bavariensis* and *B. garinii*. Often, Ixodes ticks can be carriers of several *Borrelia* species: *B. burgdorferi sensu stricto*, *B. afzelii*, *B. bavariensis*, *B. valaisiana* and *B. garinii*, – this is indicated by studies of *Ixodes* ticks removed from dogs in various European countries. Therefore, we should not rule out the possibility that ticks carrying *Borrelia* can infect animals and humans by several genospecies of *B. burgdorferi s.l.* (Hovius et al., 1998; Claerebout et al., 2013; Geurden et al., 2018). According to the respondents, in 91.6% of cases, the veterinarians observed clinical manifestations of Lyme borreliosis in the form of arthritis, so we assume that in Ukraine, the vast majority of dogs are infected by *B. burgdorferi sensu stricto* genotype, which causes the development of arthritis accompanied by lameness, joint swelling, and arthralgia with general malaise. Also, the veterinarians in 37.6% of cases observed immunopathological consequences of borreliosis infection – Lyme nephritis. Scientists associate this symptom complex not with certain genospecies of *Borrelia*, but rather with damage to the kidney tissues by immune complexes of the *Borrelia* antigen with specific antibodies (Littman et al., 2013). Somewhat less often, in 33.6% of the cases, the veterinarians noted signs of neuroborreliosis. In this case, we assume that the dogs were infected by the *B. garinii* genotype, which is associated with the damage to the nervous system. However, the manifestations of neuroborreliosis in dogs are not well documented (Littman et al., 2018). Erythema migrans is a characteristic symptom of Lyme borreliosis in humans. It is believed that it does not occur in dogs (Day et al., 2016). Based on the survey data, in 15.3% of the cases, the veterinarians noted the presence of erythema at the site of a tick bite when examining the dogs. This may be due to the fact that superficial skin excoriation, local allergic reaction, and alopecia may develop after a tick bite as a result of infection, but the possibility that the animals were infected by *B. afzelii* genotype should also be considered. The clinical symptoms of Lyme carditis were reported by only four respondents. Heart damage in dogs has been reported very rarely but was described in scientific sources (Raya et al., 2010; Day et al., 2016).

The treatment of dogs with Lyme borreliosis is based on combating the infection and mitigating pain syndrome. Usually, dogs suffering Lyme borreliosis show a quick reaction to antibiotics, within 1–2 days. Beta-lactam and tetracycline antibiotics are considered effective antimicrobial agents for the treatment of LB in dogs (Wormser et al., 2011). In general, for the treatment of LB, a course of antibiotics is prescribed for 3–4 weeks with daily oral administration. Experts recommend doxycycline as the first-line treatment for dogs with suspected Lyme disease due to its ease of administration, anti-arthritic and anti-inflammatory properties, and effectiveness in treating co-infections such as anaplasmosis, ehrlichiosis, and leptospirosis (Littman et al., 2006). Experimental studies demonstrate the successful use of cephalosporin antibiotics (cefovecin) for LB in dogs with a two-time administration in the dose of 8 mg/kg (Wagner et al., 2015). There are currently no approved veterinary antimicrobials for the treatment of LB in dogs (Littman et al., 2006). The data from survey among veterinarians in Ukraine demonstrate that in most cases, tetracycline antibiotics (Doxycycline) were used to treat Lyme disease in dogs, as reported by 79.9% of the respondents, while only 18.6% of the veterinarians used cephalosporin antimicrobials (Cefix, Ceftriaxone). In general, the data on antimicrobial use are consistent with the global practice of treatment of Lyme disease in animals. Some veterinarians also used antibiotics from the groups of macrolides (14.6%), penicillins (10.6%), aminoglycosides (8.0%), carbapenems (2.6%), and lincosamides (0.4%).

Regarding the dynamics of the incidence of Lyme borreliosis in dogs, almost half of the surveyed respondents (48.0%) noted an increase in the frequency of cases of LB in dogs. The largest number of reports on the increase in the frequency of cases of LB in dogs were received from the northern and central regions of Ukraine: the city of Kyiv (94%), the Cherkasy and Lviv regions (82% each), followed by 70% to 50% of the respondents from Khmelnytskyi, Kyiv, Chernihiv, Sumy, Ternopil, Poltava, Vinnytsia, and Kharkiv regions. The respondents from two western regions of Ukraine: Chernivtsi and Volyn regions, as well as from four regions of the southern territory of Ukraine from Mykolayiv, Odesa, Kherson, and Zaporizhia regions, did not report an increase in cases of LB in dogs. The respondents' observations regarding the dynamics of the incidence of Lyme borreliosis in dogs correspond to the results of the analysis of the prevalence of LB in dogs in the regions of Ukraine.

Our research includes the data provided to us by the veterinary practitioners and not our own research. We cannot verify the authenticity of the data received. We are unable to determine whether the sample size is sufficient, since it unknown how many veterinarians registered in Ukraine are conducting clinical veterinary practice and what is the total population of domestic dogs. The obtained data are uneven in the time period and across the regions of Ukraine. We do not have data on the success of treatment of dogs with LB using different groups of antimicrobial drugs. The data do not reflect an epizootic situation, but, regardless of the shortcomings of the conducted research, it reveals the general trends regarding the prevalence of Lyme borreliosis in Ukraine, clinical manifestations, diagnosis, and treatment of Lyme disease.

Conclusions

The analysis of survey data indicates that the veterinarians diagnosed Lyme borreliosis only in dogs, and in most cases (91.6%), Lyme disease in dogs manifested as Lyme arthritis. Veterinarians have not reported LB in other species of animals. Lyme borreliosis in dogs is recorded throughout Ukraine, from 0.0 to 3.0 cases per year in different regions. As a result of a comparative geographical and statistical analysis of the data on the incidence of LB in dogs, we determined that the dynamics of the prevalence of Lyme disease are influenced by complexes of natural physical and geographical factors (natural zones) and individual climatic indicators, such as annual air temperature, soil surface temperature and gross moisture of the territory. The most optimal conditions for the circulation of the causative agent of LB were observed in the natural zones of the forest-steppe and broad-leaved forests, where the highest incidence rates of LB in dogs were recorded, from 1.02 to 1.22 cases of LB per year, respectively. Also, we determined that in the territories with annual average air temperature in the range of 6 to 8 °C, the soil surface July temperature of 22 to 26 °C, and gross moisture in the range from 500 to >650 mm, the incidence Lyme borreliosis in dogs was comparatively higher than in the territories where the climatic indicators were lower or higher ($P < 0.001$). The territories with the highest rates of borreliosis in dogs (average of 0.84–1.57 cases of borreliosis per year) in descending order include northern, central, western, and eastern regions. The territories with unfavourable climatic conditions for the circulation of the causative agent of Lyme disease include the southern territories of the steppe natural zone of Ukraine, which are characterized by low gross moisture (<450 mm), high-temperature indicators of the soil surface (from 26 to >28 °C) and high average annual air temperature (10 to 12 °C). Also, as a result of the survey, we determined what methods are used by veterinary doctors in Ukraine to diagnose and treat LB in dogs. The majority of respondents reported the use of immunochromatographic analysis to diagnose LB in dogs (31.4%). Those data are alarming because, as noted in a number of scientific sources, the use of only serological methods cannot definitively diagnose LB, but only confirms the contact of the animal with the *Borrelia* antigen. For the treatment of dogs diagnosed with Lyme disease, the veterinarians in their practice more often used Doxycycline – an antibiotic of the tetracycline group, which is consistent with the global practice of treatment of LB in dogs. Almost half of the interviewed veterinarians (48.0%; 137 out of 285) reported an increase in the frequency of cases of Lyme borreliosis in dogs, which raises concerns about the possible complication of the epizootic situation regarding Lyme borreliosis, especially in areas where there are all prerequisites for maintaining the circulation of the pathogen – *B. burgdorferi s.l.*, both in natural and urban environments. Also, trends in the increasing frequency of LB cases in dogs should forewarn not only veterinarians but also doctors, since *Borrelia* has zoonotic potential.

The authors declare that they have no conflict of interest.

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