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Ecology and prevalence of *Borrelia burgdorferi* s.l. in *Ixodes ricinus* (Acari: Ixodidae) ticks

IVANA IVANOVIĆ[1*], MARINA ŽEKIĆ STOŠIĆ[2], EVA RUŽIĆ SABLJIĆ[3], TJAŠA CERAR KIŠEK[3], VESNA CVITKOVIĆ ŠPIK[3], ALEKSANDRA POPOVIĆ[1] and SARA SAVIĆ[2]

[1] University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000, Novi Sad, Serbia

[2] Scientific Veterinary Institute 'Novi Sad', Novi Sad, Serbia

[3] Laboratory for Diagnostics of Borreliosis and Leptospirosis, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

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RESEARCH ARTICLE

ABSTRACT

Weather conditions greatly affect tick population densities and activity, on which depends the occurrence of tick-borne diseases (TBDs). During the spring months from 2017 to 2019, 1,357 specimens of *Ixodes ricinus* ticks were collected at 9 localities in the vicinity of Novi Sad (Serbia). The number of collected ticks varied considerably among the different sampling sites and years. Also, a statistically significant difference was found between months and observed number of ticks for each stadium. By statistical analysis of tick activity depending on microclimatic conditions, a positive and statistically significant relationship between temperature and the number of ticks for each life stage was established, but not for humidity. Dew had a statistically significant impact only on nymphs but not on adults. The infection rate of *Borrelia burgdorferi* s.l. was the highest in March (46.5–51.2%) and the lowest in May (32.9–34.8%). The highest prevalence was detected in males and the lowest in nymphs. Since there is a positive and statistically significant correlation between tick number and prevalence, the ability to provide weather-based predictions of the seasonal patterns of current tick activity is important for the risk assessment of TBDs such as Lyme borreliosis.

KEYWORDS

Ixodes ricinus, microclimate, tick activity, *Borrelia burgdorferi*, prevalence

INTRODUCTION

Tick-borne diseases (TBDs) are among the most important zoonoses in the world. In recent years, a large number of studies have been published referring to the bio-ecology and vector potential of ticks and the expansion of TBDs in Europe (Fuente, 2018; Heylen et al., 2019; Kurokawa et al., 2020; Garcia-Vozmediano et al., 2020). *Ixodes ricinus* is a primary tick species of human concern in Europe. This species is a confirmed vector and/or reservoir of various infectious pathogens, such as viruses (tick-borne encephalitis virus, TBEV), bacteria (*Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum, Rickettsia* spp.), and protozoa (*Babesia* spp.). *I. ricinus* has a wide geographic range; however, the spatial distribution, seasonal dynamics and population densities depend on various biotic and abiotic factors. Consequently, it is difficult to accurately define and calculate its potential risk index in the transmission of TBDs.

The most significant factors that affect tick population densities and diurnal activity are climatic (especially microclimatic) conditions (Gray et al., 2009), the diversity and density of potential host species, vegetation structure and anthropogenic influence (Estrada-Peña and

*Corresponding author.
E-mail: ivana.ivanovic@polj.uns.ac.rs

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Fuente, 2014; Uspensky, 2014). The highest *I. ricinus* density has been observed in forest ecosystems, especially in deciduous forests with rich shrubby and grassy vegetation. The highest activity in temperate continental climate is from March till November, with the main density peak in spring and the second one in late summer and early autumn. Therefore, the occurrence of TBDs has a seasonal character influenced by weather conditions as well (Gray et al., 2009). Since the density and questing activity of ticks are seasonally and spatially variable, it is more than necessary to continuously monitor the population dynamics, especially in their natural habitats.

Lyme borreliosis (LB), caused by spirochaetes of the *B. burgdorferi* s.l. complex, is the most commonly reported human TBD in Europe. According to Mysterud et al. (2018), about 65,000 new cases are annually registered in Europe, and 300,000 in the United States. At least five species have been described as the causative agents of human Lyme borreliosis: *B. burgdorferi* sensu stricto*, Borrelia afzelii, Borrelia garinii, Borrelia bavariensis, Borrelia spielmanii* (Potkonjak et al., 2016). *Borrelia lusitaniae*, *Borrelia valaisiana* and *Borrelia bassettii* have been isolated from human blood, although their pathogenic potential is still unclear (Ćakić et al., 2019).

Therefore, the aim of this study was to clarify the influence of meteorological parameters (temperature, humidity and dew) on tick density in the spring months, and to determine the prevalence of *B. burgdorferi* s.l. infected ticks collected from nature.

MATERIALS AND METHODS

Prospected localities

The study was conducted at 9 localities: Andrevlje (N45 10.268 E19 38.496), Testera (N45 10.534 E19 38.596), Letenka (N45 13.719 E19 68.428), Jabuka (N45 13.557 E19 69.221), Brankovac (N45 15.704 E19 74.830), Zmajevac (N45 09.321 E19 46.508), Crni čot (N45 15.223 E19 83.884), and Stražilovo (N45 17.003 E19 91.243) on the Fruška gora mountain (Vojvodina, Serbia), and Kać (N45 17.667 E19 54.019, the Institute of Poplar Novi Sad experimental field). All chosen localities represent natural tick habitats, forest ecosystems fragmented with sporadic areas covered with herbaceous plants, ground shrubby vegetation, and meadows. The diverse compositions of phytocoenoses provide favourable habitats for many vertebrate species preferably parasitised by ticks. Besides their exceptional biodiversity features, the localities were also selected according to the frequency of human activities, as they represent landscaped popular picnic areas, cycling and hiking trails, or recreational centres visited annually by a large number of children and people.

Tick collection, storage and identification

Ticks were collected during a three-year research period (2017–2019), twice a month from March till May. The sampling activity was determined by the meteorological parameters, and was not conducted when temperatures were below 5 °C and in case of rain. Ticks were collected using a 1-m^2 white flannel cloth (Dantas-Torres et al., 2013). At each site, two 100-m-long transects were chosen and prospected for tick presence for 30 min each. Both sides of the cloth were inspected five times, i.e., every 20 m (Maupin et al., 1991).

Ticks were collected in plastic tubes with a cotton piece soaked in water to maintain the necessary humidity and prevent tick desiccation. The tubes were closed with perforated plastic stoppers to obtain sufficient ventilation, all with the aim to transport them alive to the laboratory for further molecular analyses. Ticks were identified up to species according to Estrada-Peña et al. (2017). Only *I. ricinus* adults and nymphs were isolated for further molecular analyses.

Meteorological parameters

Air temperature (T, °C) and relative air humidity (H, %) were measured using a thermo-hygrograph placed on the ground level during tick sampling at each site at 2 PM. The detailed data for dew were recorded by observatories of the Hydrometeorological Service of the Republic of Serbia placed in the vicinity of the prospected localities.

Molecular analyses of *B. burgdorferi* using PCR

In order to determine tick infection with *B. burgdorferi* s.l., nymphs, females and males of *I. ricinus* were separated. The presence of *B. burgdorferi* s.l. was determined by real-time qPCR test, targeting the *RecA* gene, which was previously proven as a good genetic marker for *Borrelia* presence (Žekić Stošić et al., 2021). After sample preparation, ticks were washed in alcohol and rinsed in water. DNA was extracted from whole tick tissues according to the manufacturer's protocol (Genesig Easy extraction kit, Primedesign Ltd). DNA extraction was a six-step process, and sample lysis was stimulated by incubation with lysis buffer and proteinase K. This was followed by the addition of binding buffer and magnetic beads. At the end of the process, high-purity DNA was eluted which was used directly in reactions as a sample. The detection of the presence of a target DNA was done by real-time quantitative PCR, using Lyme disease Genesig® Easykit. This kit detects a target gene (the *RecA* gene) which is a good genetic marker for three *Borrelia* species (*B. afzelii*, *B. garinii* and *B. burgdorferi* s.s.). The detection of targeted DNA presence for confirmation was done by real-time PCR Light Mix Modular *Borrelia* spp. kit (TIB MolBiol) (Cerar et al., 2019).

Prevalence calculation

The prevalence of *B. burgdorferi* s.l. was calculated according to the following formula:

$$P(\%) = B/C \times 100,$$

where:

B – the number of ticks infected with *B. burgdorferi* s.l.
C – the total number of ticks analysed

Statistical analyses

Numerical variables were summarised with mean, median, standard deviation, minimum and maximum, while categorical variables were analysed using frequencies. Kolmogorov–Smirnov and Shapiro–Wilk normality tests were used to test the normality of variables. Spearman rank correlation was applied to measure the degree of association between two variables. In order to compare the medians of two or more independent groups, Mann–Whitney *U* test and Kruskal–Wallis test were conducted. The statistical analyses were performed using SPSS ver. 25.

The tick number represented the collected specimens during the study, and it is a non-negative integer valued variable. On the other hand, the prevalence represented the ratio of infected ticks out of all observed ticks, so it was used as a variable with range values [0, 100%]. Therefore, the prevalence statistical modelling included its transformation into binary variable taking value 1, if the original value was higher than the prevalence variable mode, otherwise taking value 0:

$$x = \begin{cases} 0, & \text{prevalence} \le 0 \\ 1, & \text{prevalence} > 0 \end{cases}.$$

The correlation of dependent (tick number and prevalence) and the set of independent variables (temperature, humidity and dew) was statistically analysed and modelled. To analyse the correlation of tick number and meteorological parameters the negative binomial regression model (NBR) was used (Moghimbeigi, 2015). NBR models overcome the over-dispersion since the expected tick frequency (μ_i) was set up as a function of the independent variables plus gamma-distributed error term $\varepsilon_i \sim Gamma(1, \alpha)$ to relax the Poisson regression assumption of equal mean and variance by including additional randomness:

$$\ln(\mu_i) = x_i^T \beta + \varepsilon_i.$$

The NBR distribution mean was μ_i, the variance was $\mu_i + \alpha\mu_i^2$ and α was the over-dispersion parameter. Regarding previous, dependent variable variation analyses were described as the following models:

$$\ln(Num\ of\ male\ ticks) = \beta_0 + \beta_1{*}(Temp \times Hum) + \beta_2{*}(year) + \beta_3{*}(dew) + \beta_4{*}(wind)$$

$$\ln(Num\ of\ female\ ticks) = \beta_0 + \beta_1{*}(Temp \times Hum) + \beta_2{*}(year) + \beta_3{*}(dew) + \beta_4{*}(wind)$$

$$\ln(Num\ of\ nymphs) = \beta_0 + \beta_1{*}(Temp \times Hum) + \beta_2{*}(year) + \beta_3{*}(dew) + \beta_4{*}(wind)$$

NBR model results were presented with: estimated coefficients (B), standard errors (SE), confidence interval of the estimated coefficients (95% CI), significance of χ^2 tests (*P*), and incidence rate ratios (IRR). Omnibus test confirmed validity of the models (males: $\chi^2 = 23.402$, $P = 0.000$, females: $\chi^2 = 18.813$, $P = 0.001$, nymphs: $\chi^2 = 30.320$, $P = 0.000$) (Fávero and Belfiore, 2019).

RESULTS AND DISCUSSION

Tick abundance

During the study period, 1,357 *I. ricinus* specimens were collected from prospected localities: 336 nymphs, 545 females and 476 males (Fig. 1). The highest number of nymphs was noticed in April, contrary to females and males

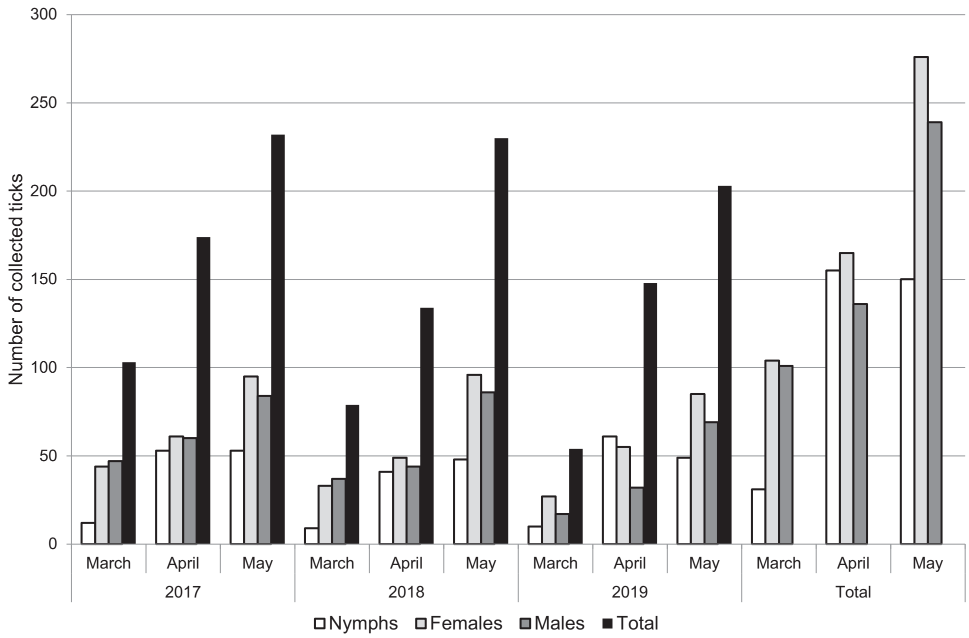

Fig. 1. Number of collected *Ixodes ricinus* nymphs, females and males considering month and year

which were more abundant in May. The statistical analyses detected a positive and statistically significant relationship of medium strength between month and observed tick number for each stadium (male: $\rho = 0.333$, $P = 0.002$; female: $\rho = 0.391$, $P = 0.000$; nymphs: $\rho = 0.491$, $P = 0.000$). The correlation was strongest for nymphs, and the weakest for males. These results are in accordance with those published by Schulz et al. (2014) who also detected nymph and adult activity peak in late April and May, respectively.

The highest *I. ricinus* population was noticed in 2017 (Fig. 1). Highly significant statistical difference was calculated for collected tick medians comparing different months and years (2017: $\chi^2 = 9.996$, $P = 0.007$; 2018: $\chi^2 = 10.691$, $P = 0.005$; 2019: $\chi^2 = 17.839$, $P = 0.000$). There was no significant difference in tick presence among the three years. The statistical analyses emphasised that nymph activity depended on the month in an additive way. Similar results were observed by Hansford et al. (2017) for southern England, where tick presence was significantly greater during spring and summer than during autumn, although nymphs were active during all seasons, while their intensity during spring was significantly higher. The same conclusion for *I. ricinus* nymphs was published by Cat et al. (2017) for three Western European countries (The Netherlands, Belgium, and France).

Highly significant statistical difference was calculated for collected tick medians comparing different localities and each year (tick number, medians, χ^2 and P values are presented in Table 1).

The population density of questing *I. ricinus* nymphs and adults varied considerably among the different sampling sites and years. The highest tick abundance was recorded at Stražilovo, which was distinguished by dense deciduous forest, fragmented with few shrubby and herbaceous plants areas. These are the most favourable microclimatic conditions for the population growth and maintenance of all ixodid tick species, but it also provides an excellent habitat for a variety of vertebrate host species. Localities with lower tick abundance, such as Zmajevac, Letenka and Jabuka, differed in their vegetation structure from the other sampling sites. There was a small amount or no leaf litter, where the very thin herbaceous ground vegetation was unable to retain enough humidity. Therefore, low tick abundance was continuously recorded, which is in accordance with the results reported by Schulz et al. (2014). This highlights the importance of local vegetation structure, which plays a crucial role in maintaining a suitable microclimatic environment and necessary tick shelter, as ticks are extremely dependent on humidity in their microhabitat.

Correlations of tick activity and climate conditions

The highest temperature was recorded in May 2017 (27.0 °C, Stražilovo), and humidity in March 2018 (96.0%, Stražilovo). There were no recorded extremes, so the measured meteorological parameters were in accordance with the multi-annual averages of meteorological parameters, and reflected the conditions of the temperate continental climate, typical for this area.

The average temperature and relative air humidity at prospected sites are given in Table 2. The average temperatures calculated for all years varied from 14.9 to 17.8 °C (Crni Čot and Kać, respectively), and the average humidity from 49.1 to 67.3% (Letenka and Andrevlje, respectively).

There was a positive and statistically significant correlation of medium strength between measured temperature and tick number for each stadium (male: $\rho = 0.285$, $P = 0.010$; female: $\rho = 0.345$, $P = 0.002$; nymphs: $\rho = 0.451$, $P = 0.000$). The correlation was strongest for the nymphs and the weakest for the males. Hence, there was no statistically significant correlation between the measured humidity and the tick number for any life stage. The analyses of dew influence on tick abundance highlighted a statistically significant impact only on nymphs (Mann–Whitney $U = 538$, $P = 0.009$), but not on adults. In order to test the significance of the obtained correlation regarding the influence of climate conditions on the number of collected ticks, NBR analysis was applied. For each NBR model, the estimated coefficients of temperature and humidity were positively statistically significant. The tick abundance of all life stages is likely to be higher when both temperature and humidity are elevated. The appearance of dew statistically influenced only the

Table 1. Descriptive statistics of collected ticks at each site

Location	Median			Minimum			Maximum			Total			Sum
	2017	2018	2019	2017	2018	2019	2017	2018	2019	2017	2018	2019	
Andrevlje	5	4	10	1	1	1	14	13	19	50	47	85	182
Brankovac	3	5	2	0	1	0	15	17	16	52	63	38	153
Crni Čot	6	3	2	1	0	0	11	7	7	56	27	22	105
Jabuka	2	3	1	0	0	0	8	11	10	26	31	24	81
Kać	10	6	5	3	2	2	13	10	15	79	55	61	195
Letenka	2	3	3	1	0	0	7	5	8	26	23	31	80
Stražilovo	19	11	3	1	1	1	31	35	20	155	138	63	356
Testera	5	6	5	1	0	1	18	12	20	58	51	63	172
Zmajevac	0	1	2	0	0	0	2	3	4	7	8	18	33
χ^2	34.887	28.735	19.658										
P	0.000	0.0004	0.012										

Table 2. Average temperature (T, °C) and relative air humidity (H, %) measured at particular localities and years

Locality	2017		2018		2019	
	T	H	T	H	T	H
Andrevlje	14.97	70.00	15.40	67.33	15.03	64.67
Brankovac	16.17	54.33	16.37	72.67	17.67	46.33
Crni Čot	15.90	67.33	13.63	62.33	15.13	61.33
Jabuka	17.10	52.33	17.60	63.00	15.73	52.00
Kać	17.07	52.00	18.33	57.67	17.87	39.33
Letenka	16.27	50.33	17.87	63.67	15.87	33.33
Stražilovo	16.47	58.33	18.30	70.67	18.40	57.00
Testera	16.32	68.33	14.98	63.00	15.10	58.00
Zmajevac	17.80	48.27	13.80	66.00	15.43	46.00

females, also in a direct positive correlation, although at 10% statistical level (Table 3).

Similar results were reported by Borşan et al. (2020), who calculated a significant positive correlation between the mean temperature and the total abundance of questing ticks ($\rho = 0.369$, $P < 0.01$). *I. ricinus* starts its host-seeking activity at 2.5 °C and 45% relative humidity (Hubálek et al., 2003). The collected tick number is usually strongly positively correlated to the temperature, with no, or negative correlation to the relative humidity (Jensen, 2000; Hubálek et al., 2003). Although some studies confirmed a positive statistically significant correlation (Borşan et al., 2020), Schwarz et al. (2009) found a weak negative correlation between relative humidity (5 cm above the soil surface) and the number of ticks. The absence of a statistically significant correlation between the measured humidity and the collected ticks in our study could be explained by the fact that we sampled ticks only during three spring months, when the relative air humidity was constantly high, precipitation was frequent and microclimatic conditions at each prospected locality were favourable (dense grass vegetation, long periods of dew, swollen mountain streams after winter period). According to Lindgren et al. (2000) and Estrada-Peña (2001), the abundance of *I. ricinus* depends on various abiotic and biotic factors such as temperature, humidity, vegetation type, and host density. During questing, ticks lose water through transpiration so they have to descend from the waiting point on the vegetation to the ground litter layer, where relative humidity is higher. In these microhabitats, ticks reabsorb water from the subsaturated air thanks to the ability to actively secrete and then reingest hygroscopic fluid before they can continue questing for the adequate host (Kahl and Knulle, 1988). The level of resistance of ticks to desiccation, especially among different life stages, depends on different body mass to surface ratios (Mejlon and Jaenson, 1997) and reserve materials (Hauser et al., 2018). Undoubtedly, *I. ricinus* ticks use their lipid reserve materials to quest for hosts on vegetation and to maintain their body water homeostasis for some time. The lipid consumption rate increases under unfavourable microclimatic conditions,

Table 3. The results of Negative Binomial Regression models regarding statistical correlation between the number of collected ticks of different life stages and meteorological parameters

Stadium	Variable	B	S. E.	95% CI		*P*	IRR
				Lower	Upper		
males	Intercept	−1.174	0.6335	−2.416	0.068	0.064	0.309
	Temperature	0.099	0.0219	0.056	0.142	0.000*	1.104
	Humidity	0.018	0.0077	0.003	0.033	0.017*	1.018
	Dew	0.102	0.2194	−0.328	0.532	0.643	1.107
	(Negative binomial)	0.532	0.1100	0.355	0.798		
females	Intercept	−1.211	0.7699	−2.720	0.299	0.116	0.298
	Temperature	0.074	0.0265	0.022	0.126	0.005*	1.077
	Humidity	0.021	0.0078	0.006	0.036	0.007*	1.021
	Dew	0.449	0.2445	−0.030	0.928	0.066*	1.567
	(Negative binomial)	0.623	0.1542	0.384	1.012		
nymphs	Intercept	−0.198	0.5825	−1.340	0.944	0.734	0.820
	Temperature	0.074	0.0207	0.034	0.115	0.000*	1.077
	Humidity	0.014	0.0071	3.636E-05	0.028	0.049*	1.014
	Dew	−0.024	0.2123	−0.440	0.392	0.910	0.976
	(Negative binomial)	0.537	0.1127	0.356	0.810		

Statistically significant *P* values are marked with '*'.
B – estimated coefficients; S. E. – standard error; CI – confidence interval of the estimated coefficients; *P* – significance of χ^2 tests; IRR – incidence rate ratios.

especially temperature and humidity (Randolph and Storey, 1999). According to Randolph et al. (2002), nymphs collected in spring had less fat reserves than those sampled in autumn, which confirms the vulnerability of nymphs to saturation deficit (Herrmann et al., 2013).

The correlation between tick abundance and meteorological parameters has been confirmed by numerous authors, such as Jackson et al. (1996), Randolph and Storey (1999), Perret et al. (2000), Tagliapietra et al. (2011), Schulz et al. (2014) and Osipova et al. (2017), although Humiczewska et al. (2003) did not find any such association.

The prevalence of *B. burgdorferi* s.l

Descriptive statistics of *B. burgdorferi* s.l. prevalence in ticks, regarding the locality, year, months and tick life stages are shown in Table 4. The lowest prevalence was recorded in 2019 (36.222%), in 2018 it was 41.351%, and the highest value of prevalence was calculated in 2017 (42.063%).

The prevalence of *B. burgdorferi* s.l. was found to be significantly higher during the spring months, which is in accordance with data reported by Hansford et al. (2017). Savić et al. (2010) found that the seroprevalence of *B. burgdorferi* s.l. infected ticks in the territory of Vojvodina was 22.12%, depending on the locality and the year. Similar prevalence in the same geographical region was reported by Potkonjak et al. (2016) who found *Borrelia* spp. in 21.13% of the analysed ticks collected from nature and dogs, including *B. lusitaniae* (11.3%), *B. afzelii* (7%), *B. valaisiana* (1.4%), *B. garinii* (1.4%), and *Borrelia miyamotoi* (1.4%).

There are highly significant statistical differences in the means of calculated *B. burgdorferi* s.l. prevalence and locality summarised for each prospected year, as well as among life stages ($\chi^2 = 9.812$, $P = 0.007$). There was a positive and statistically significant correlation between the number of collected ticks and the prevalence of *B. burgdorferi* s.l. calculated for females ($\rho = 0.343$, $P = 0.002$) and nymphs ($\rho = 0.476$, $P = 0.000$). The correlation coefficient sign in case of males was also positive, but it was not statistically significant and the strength was low.

Comparing different life stages, the highest prevalence of *B. burgdorferi* s.l. has been confirmed in males, which is in accordance with the findings of Wielinga et al. (2006) who found that females had lower levels of *Borrelia* infection than males (5.2% versus 8.3%). As opposed to thesed data, Remesar et al. (2019) detected *B. burgdorferi* s.l. in 11.84% of *I. ricinus* ticks, and the prevalence was significantly higher in females, which had also been reported in earlier studies carried out throughout Europe (Sormunen et al., 2016; Espí et al., 2017).

The prevalence values of *B. burgdorferi* s.l. can vary significantly depending on the locality (Hornok et al., 2017) because of the complexity of *B. burgdorferi* s.l. occurrence, maintenance, and cycle. Besides ticks, different animal species are included in the epizootiology, especially small mammals that are suitable as *Borrelia* reservoirs (Sprong et al., 2009). The presence and spatial distribution of particular vertebrate species can play a significant role in maintaining tick populations as vectors, maintaining and transmitting the pathogens, spreading of risk areas, and transmitting pathogens to new habitats (Trout Fryxell et al., 2015). Unlike rodents, deer and roe deer, on which *I. ricinus* often parasitises, are not suitable for maintaining *Borrelia* (Hornok et al., 2017). In habitats where the number of deer (Cervidae) is lower, the natural foci of *Borrelia* are often recorded (hotspots), since in the absence of the large hosts, the probability that ticks will feed on rodents increases (Perkins et al., 2006). This could explain the significantly higher mean prevalence found at localities that are fragmented or more urban (Kać, Stražilovo, Andrevlje, Brankovac, Testera) and often visited by hikers and recreationists, because these activities disturb ungulates, which then retreat to the less disturbed habitats. The annual variation in the tick number is related to changes in the potential host density, which in forest ecosystems also may

Table 4. Descriptive statistics of *Borrelia burgdorferi* s.l. prevalence (%) in *Ixodes ricinus* ticks regarding locality, months, year, and tick life stages

Locality	Mean			Male	Female	Nymphs
	2017	2018	2019			
Andrevlje	41.852	49.414	39.223	50.198	28.175	52.116
Brankovac	43.245	55.613	57.361	73.321	32.778	50.121
Crni Cot	43.665	54.444	26.852	41.984	67.130	15.847
Jabuka	30.754	12.020	5.000	18.519	15.922	13.333
Kac	72.485	72.738	76.085	84.815	77.315	59.178
Letenka	27.910	31.852	41.574	55.556	18.519	27.262
Stražilovo	46.528	60.001	35.703	47.396	62.046	32.790
Testera	55.460	26.821	30.309	40.617	43.547	28.426
Zmajevac	16.667	9.259	13.889	25.926	0.000	13.889
χ^2	20.560	35.320	31.509			
P	0.008	0.000	0.000			
Mean				48.704	38.381	32.551
March	51.205	46.508	47.160			
April	41.496	42.742	28.550			
May	33.488	34.805	32.955			

depend on the amount of tree seed production, an important food chain basis. According to Bregnard et al. (2021), a strong positive correlation was confirmed between the amount of beech acorn production and the number of nymphs two years later. The mass production of acorns indicates the large quantities of easily available food, so the small mammal populations increase during the subsequent year. This also has an impact on the survival and reproductive success of other mammalian species. Consequently, tick larvae have an opportunity to feed on a larger number of hosts in the year after the increased acorn fruiting, and this results in an increase of nymph abundance in the following year (Ostfeld et al., 2018; Krawczyk et al., 2020). Bogdziewicz and Szymkowiak (2016) also highlighted the association between oak acorn production and the incidence of Lyme disease in humans, although the tick populations were not monitored during that study.

In conclusion, statistical analyses of tick activity depending on microclimatic conditions confirmed positive and statistically significant correlation between temperature and tick number of each life stage, but such correlation was not found between humidity and tick number. The influence of dew had a statistically significant impact only on nymphs but not on adults. According to the results obtained, there was a positive and statistically significant correlation between tick number and prevalence of *B. burgdorferi* s.l. calculated for females and nymphs, although in the case of males the correlation was also positive, but not statistically significant. Weather-conditioned changes in tick activity and abundance determine the seasonal risk of tick bites and thus of tick-borne diseases, so they could consequently be used as indicators of great interest for public health. Therefore, an ability to provide realistic, weather-based predictions of current seasonal patterns of the activity of particular tick species is a prerequisite for the development of possible risk scenarios for TBDs such as Lyme borreliosis.

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REFERENCES

Bogdziewicz, M. and Szymkowiak, J. (2016): Oak acorn crop and Google search volume predict Lyme disease risk in temperate Europe. Basic Appl. Ecol. **17**, 300–307.

Borşan, S. D., Toma-Naic, A., Péter, Á., Sándor, A., Cosmin Peştean, C. and Mihalca, A. D. (2020): Impact of abiotic factors, habitat type and urban wildlife on the ecology of hard ticks (Acari: Ixodidae) in urban and peri-urban habitats. Parasit. Vectors **13**, 476.

Bregnard, C., Rais, O. and Voordouw, M. J. (2021): Masting by beech trees predicts the risk of Lyme disease. Parasit. Vectors **14**, 168.

Cat, J., Beugnet, F., Hoch, T., Jongejan, F., Prangé, A. and Chalvet-Monfray, K. (2017): Influence of the spatial heterogeneity in tick abundance in the modeling of the seasonal activity of *Ixodes ricinus* nymphs in Western Europe. Exp. Appl. Acarol. **71**, 115–130.

Cerar, T., Meta Kodre, K., Cvitković Špik, V. and Ružić-Sabljić, E. (2019): Performance of LightMix® Modular *Borrelia* spp. on samples from official proficiency panels. Book of Abstract of International Symposium on Tick-Borne Pathogens and Disease (ITPD 2019), Vienna, Austria 8–11 September. p. 59.

Ćakić, S., Veinović, G., Cerar, T., Mihaljica, D., Sukara, R., Ružić-Sabljić, E. and Tomanović, S. (2019): Diversity of Lyme borreliosis spirochetes isolated from ticks in Serbia. Med. Vet. Entomol. **33**, 512–520.

Dantas-Torres, F., Paolo Lia, R., Capelli, G. and Otranto, D. (2013): Efficiency of flagging and dragging for tick collection. Exp. Appl. Acarol. **61**, 119–127.

Espí, A., Del Cerro, A., Somoano, A., García, V., Prieto, J. M., Barandika, J. F. and García-Pérez, A. L. (2017): *Borrelia burgdorferi* sensu lato prevalence and diversity in ticks and small mammals in a Lyme borreliosis endemic Nature Reserve in North-Western Spain. Incidence in surrounding human populations. Enferm. Infecc. Microbiol. Clín. **35**, 563–568.

Estrada-Peña, A. (2001): Distribution, abundance, and habitat preferences of *Ixodes ricinus* (Acari: Ixodidae) in Northern Spain. J. Med. Entomol. **38**, 361–370.

Estrada-Peña, A. and Fuente, J. (2014): The ecology of ticks and epidemiology of tick-borne viral diseases. Antivir. Res. **108**, 104–128.

Estrada-Peña, A., Mihalca, A. and Petney, T. (2017): Ticks of Europe and North Africa. A Guide to Species Identification. Springer Nature, Switzerland. 189 pp.

Fávero L. P. and Belfiore P. (2019): Regression models for count data: Poisson and negative binomial, Chapter 15. In: Fávero L. P. and Belfiore P. (eds) Data Science for Business and Decision Making. Academic Press, USA. pp. 617–703.

Fuente, J. D. (2018): Controlling ticks and tick-borne diseases... looking forward. Ticks Tick Borne Dis. **95**, 1354–1357.

Garcia-Vozmediano, A., Krawczyk, A., Sprong, H., Rossi, L., Ramassa, E. and Tomassone, L. (2020): Ticks climb the mountains: ixodid tick infestation and infection by tick-borne pathogens in the Western Alps. Ticks Tick Borne Dis. **11**, 101489.

Gray, J. S., Dautel, H., Estrada-Peña, A., Kahl, O. and Lindgren, E. (2009): Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. **2009**, 1–12.

Hansford, K. M., Fonville, M., Gillingham, E. L., Coipan, E. C., Pietzsch, M. E., Krawczyk, A. I., Vaux, A., Cull, B., Sprong, H. and Medlock, J. M. (2017): Ticks and Borrelia in urban and peri-urban green space habitats in a city in southern England. Ticks Tick Borne Dis. **8**, 353–361.

Hauser, G., Rais, O., Cadenas, F. M., Gonseth, Y., Bouzelboudjen, M. and Gern, L. (2018): Influence of climatic factors on *Ixodes ricinus* nymph abundance and phenology over a long-term monthly observation in Switzerland (2000–2014). Parasit. Vectors **11**, 289.

Herrmann, C., Voordouw, M. J. and Gern, L. (2013): *Ixodes ricinus* ticks infected with the causative agent of Lyme disease, *Borrelia burgdorferi* sensu lato, have higher energy reserves. Int. J. Parasitol. **43**, 477–483.

Heylen, D., Lasters, R., Adriaensen, F., Fonville, M., Sprong, H. and Matthysen, E. (2019): Ticks and tick-borne diseases in the city: role of landscape connectivity and green space characteristics in a metropolitan area. Sci. Total Environ. **670**, 941–949.

Hornok, S., Mulvihill, M., Szőke, K., Gönczi, E., Kinga S., Gyuranecz, M. and Hofmann-Lehmann, R. (2017): Impact of a freeway on the dispersal of ticks and *Ixodes ricinus*-borne pathogens: forested resting areas may become Lyme disease hotspots. Acta Vet. Hung. **65**, 242–252.

Hubálek, Z., Halouzka, J. and Juricova, Z. (2003): Host-seeking activity of ixodid ticks in relation to weather variables. J. Vector Ecol. **28**, 159–165.

Humiczewska, M., Kuźna-Grygiel, W., Kołodziejczyk, L., Białek, S., Kozłowska, A., Rozen, W. and Sych, Z. (2003): Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* population in forests of north-western Poland. Wiad. Parazytol. **49**, 255–271.

Jackson, L. K., Gaydon, D. M. and Goddard, J. (1996): Seasonal activity and relative abundance of *Amblyomma americanum* in Mississippi. J. Med. Entomol. **33**, 128–131.

Jensen, P. M. (2000): Host seeking activity of *Ixodes ricinus* ticks based on daily consecutive flagging samples. Exp. Appl. Acarol. **24**, 695–708.

Kahl, O. and Knulle, W. (1988): Water vapour uptake from subsaturated atmospheres by engorged immature ixodid ticks. Exp. Appl. Acarol. **4**, 73–83.

Krawczyk, A. I., van Duijvendijk, G. L. A., Swart, A., Heylen, D., Jaarsma, R. I., Jacobs, F. H. H., Fonville, M., Sprong, H. and Takken, W. (2020): Effect of rodent density on tick and tick-borne pathogen populations: consequences for infectious disease risk. Parasit. Vectors **13**, 34.

Kurokawa, C., Lynn, G. E., Pedra, J. H., Pal, U., Narasimhan, S. and Fikrig, E. (2020): Interactions between *Borrelia burgdorferi* and ticks. Nat. Rev. Microbiol. **18**, 587–600.

Lindgren, E., Talleklint, L. and Polfeldt, T. (2000): Impact of climatic change on the northern latitude limit and populations density of the disease-transmitting European tick *Ixodes ricinus*. Environ. Health Perspect. **108**, 119–123.

Maupin, G. O., Fish, D., Zultowsky, J., Campos, E. G. and Piesman, J. F. (1991): Landscape ecology of Lyme disease in a residential area of Westchester County, New York. Am. J. Epidemiol. **133**, 1105–1113.

Mejlon, H. A. and Jaenson, T. G. T. (1997): Questing behaviour of *Ixodes ricinus* ticks (Acari: Ixodidae). Exp. Appl. Acarol. **21**, 747–754.

Moghimbeigi, A. (2015): Two-part zero-inflated negative binomial regression model for quantitative trait loci mapping with count trait. J. Theor. Biol. **372**, 74–80.

Mysterud, A., Stigum, V. M., Seland, I. V., Herland, A., Easterday, W., Jore, S., Østerås, O. and Viljugrein, H. (2018): Tick abundance, pathogen prevalence, and disease incidence in two contrasting regions at the northern distribution range of Europe. Parasit. Vectors **11**, 309.

Osipova, T. N., Grigoryeva, L. A., Samoylova, E. P., Shapar, A. O. and Bychkova, E. M. (2017): The influence of meteorological factors on the activity of adult taiga ticks (*Ixodes persulcatus* Sch., Ixodinae) in St. Petersburg and its environs. Entomol. Rev. **97**, 554–563.

Ostfeld, R. S., Levi, T., Keesing, F., Oggenfuss, K. and Canham, C. D. (2018): Tick-borne disease risk in a forest food web. Ecology **99**, 1562–1573.

Perkins, S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. and Hudson, P. J. (2006): Localized deer absence leads to tick amplification. Ecology **87**, 1981–1986.

Perret, J. L., Guigoz, E., Rais, O. and Gern, L. (2000): Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland). Parasitol. Res. **86**, 554–557.

Potkonjak, A., Kleinerman, G., Gutiérrez, R., Savić, S., Vračar, V., Nachum-Biala, Y., Jurišić, A., Rojas, A., Petrović, A., Ivanović, I., Harrus, S. and Baneth, G. (2016): Occurrence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks with first identification of *Borrelia miyamotoi* in Vojvodina, Serbia. Vector Borne Zoonotic Dis. **16**, 631–635.

Randolph, S. E. and Storey, K. (1999): Impact of microclimate on immature tick–rodent interactions (Acari: Ixodidae): implications for parasite transmission. J. Med. Entomol. **36**, 741–748.

Randolph, S. E., Green, R. M., Hoodless, A. N. and Peacey, M. F. (2002): An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. Int. J. Parasitol. **32**, 979–989.

Remesar, S., Díaz, P., Venzal, J. M., Prieto, A., Estrada-Peña, A., López, C. M., Panadero, R., Fernández, G., Díez-Baños, P. and Morrondo, P. (2019): Longitudinal study of infection with *Borrelia* spp. in questing ticks from North-Western Spain. Vector Borne Zoonotic Dis. **19**, 785–792.

Savić, S., Vidić, B., Lazić, S., Lako, B., Potkonjak, A. and Lepsanović, Z. (2010): *Borrelia burgdorferi* in ticks and dogs in the province of Vojvodina, Serbia. Parasite **17**, 357–361.

Schulz, M., Mahling, M. and Pfister, K. (2014): Abundance and seasonal activity of questing *Ixodes ricinus* ticks in their natural habitats in southern Germany in 2011. J. Vector Ecol. **39**, 56–65.

Schwarz, A., Maier, W. A., Kistemann, T. and Kampen, H. (2009): Analysis of the distribution of the tick *Ixodes ricinus* L. (Acari: Ixodidae) in a nature reserve of western Germany using Geographic Information Systems. Int. J. Hyg. Environ. Health **212**, 87–96.

Sormunen, J. J., Klemola, T., Vesterinen, E. J., Vuorinen, I., Hytönen, J., Hänninen, J., Ruohomäki, K., Sääksjärvi, I. E., Tonteri, E. and Penttinen, R. (2016): Assessing the abundance, seasonal questing activity, and Borrelia and tick-borne encephalitis virus (TBEV) prevalence of *Ixodes ricinus* ticks in a Lyme borreliosis endemic area in Southwest Finland. Ticks Tick Borne Dis. **7**, 208–215.

Sprong, H., Wielinga, P. R., Fonville, M., Reusken, C., Brandenburg, A. H., Borgsteede, F., Gaasenbeek, C. and van der Giessen, J. W. (2009): *Ixodes ricinus* ticks are reservoir hosts for *Rickettsia helvetica* and potentially carry flea-borne *Rickettsia* species. Parasit. Vectors **2**, 41.

Tagliapietra, V., Rosà, R., Arnoldi, D., Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H. C. and Rizzoli, A. (2011): Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinus* (Acari, Ixodidae) in Italy. Vet. Parasitol. **183**, 114–124.

Trout Fryxell, R. T., Moore, J. E., Collins, M. D., Kwon, Y., Jean-Philippe, S. R., Schaeffer, S. M., Odoi, A., Kennedy, M. and Houston, A. E. (2015): Habitat and vegetation variables are not enough when predicting tick populations in the southeastern United States. PLoS One **10**, e0144092.

Uspensky, I. (2014): Tick pests and vectors (Acari: Ixodoidea) in European towns: introduction, persistence and management. Ticks Tick Borne Dis. **5**, 41–47.

Wielinga, P. R., Gaasenbeek, C., Fonville, M., de Boer, A., de Vries, A., Dimmers, W., Akkerhuis Op Jagers, G., Schouls, L. M., Borgsteede, F. and van der Giessen, J. W. (2006): Longitudinal analysis of tick densities and *Borrelia, Anaplasma*, and *Ehrlichia* infections of *Ixodes ricinus* ticks in different habitat areas in The Netherlands. Appl. Environ. Microbiol. **72**, 7594–7601.

Žekić Stošić, M., Tomanović, S., Sukara, R., Milošević, S. and Savic, S. (2021): Detection of *Borrelia* spirochetes in ticks with q16 Real-Time PCR. Arch. Vet. Med. **14**, 85–98.