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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

The influence of local environmental factors in southwestern Poland on the abundance of *Ixodes ricinus* and prevalence of infection with *Borrelia burgdorferi* s.l. and *B. miyamotoi*

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Abstract

Ticks are important ectoparasites and vectors of pathogens that cause disease in humans and animals. The natural habitat of *Ixodes ricinus* ticks is forests, which are convenient habitats to search for hosts, including reservoir hosts, and therefore can be an important habitat source of tick-borne pathogens. The aim of the study was to assess the usefulness of detailed forest habitat-type maps to estimate the tick-borne risk at a local scale (Lower Silesia, SW Poland). For the purposes of estimating tick abundance, we used the land cover maps available from the Forest Data Bank. For *I. ricinus* collection, nine sites located in three forest habitat types were chosen: broadleaf forest, mixed broadleaf and coniferous forest and coniferous forest. Ticks were collected once a month from April to June 2018 and 2019 using the standard flagging method. At each of the nine sites, ticks were collected in four plots, of 100 m^2 each. Tick abundance was analysed using general linear mixed models (GLMM). A total of 2196 (10.1/100 m^2) ticks were collected, including 2093 *Ixodes ricinus* (95.3%; 9.6/100 m^2), 46 *Dermacentor reticulatus* (2.1%; 0.2/100 m^2) and 57 *Haemaphysalis concinna* (2.6%; 0.3/100 m^2). Among the collected *I. ricinus* were 589 larvae (28.1%; 2.7/100 m^2), 1261 nymphs (60.3%; 5.8/100 m^2), 128 females (6.1%; 0.6/100 m^2) and 115 males (5.5%; 0.5/100 m^2). We found a highly significant effect of forest habitat type on the density of ticks for broadleaf forest (coefficient = 1.87267, *p*-value = 2.79e − 07). Additionally, a significant influence of air temperature and relative humidity on the abundance of ticks was observed. During spring, the peak activity of *I. ricinus* was recorded in May and June. For DNA amplification of *Borrelia burgdorferi* s.l., a nested PCR method was used. Out of 494 *I. ricinus*, 83 (16.8%) were positive for *Borrelia* spp. The RFLP method showed the occurrence of five species including four belonging to the *B. burgdorferi* s.l. complex: *B. afzelii* (30.1%), *B. garinii* (38.6%), *B. valaisiana* (2.4%) and *B. lusitaniae* (18.1%). Furthermore, *B. miyamotoi* (9.6%), a species belonging to bacteria that cause relapsing fever as well as co-infection of *B. miyamotoi*/*B.lusitaniae* (1.2%) were found. The differences in the infection level of *Borrelia* spp. between broadleaf forest and mixed broadleaf and coniferous forest were statistically significant.

Keywords *Ixodes ricinus* · *Borrelia burgdorferi* s.l. · *Borrelia miyamotoi* · Forest habitat types · Lower Silesia (SW Poland)

Introduction

In Central Europe, *Ixodes ricinus* is one of the most important tick species from a medical and veterinary point of view, because of its direct pathogenic effect and ability of pathogen transmission (Nowak-Chmura and Siuda 2012; Petney et al. 2012; Medlock et al. 2013; Kahl 2018; Parola and Paddock 2018). All developmental stages can potentially feed on humans; however, nymphs and females are the most common (Liebisch et al. 1998; Wilhelmsson et al. 2013; Karbowiak et al. 2015). The risk group is composed of forest workers, farmers, hunters and people visiting forests for recreational purposes (Cisak et al. 2005; Rizzoli et al.

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2011; Kmieciak et al. 2016; Kowalec et al. 2017). The most common recorded tick-borne disease in the northern hemisphere is Lyme disease (LD) caused by the spirochetes of the *Borrelia burgdorferi* s.l. complex (Medlock et al. 2018). The recorded increase in LD cases in Europe is related to an increase in the abundance and enlargement of the geographical range distribution of *I. ricinus*, a greater public awareness of tick-borne diseases, and the improvement of diagnostic methods (Dantas-Torres et al. 2012; Jaenson et al. 2012; Medlock et al. 2013, 2018; Wikel 2018). In 2019, the annual incidence rate of LD was 53.7/100,000 inhabitants in Poland and 30.9/100,000 in Lower Silesia. (www.pzh.gov.pl).

The environmental conditions influencing the abundance of ticks and the prevalence of their infection are an important indicator of LD risk (Rousseau et al. 2017). Factors affecting the occurrence and activity of *I. ricinus* include primarily vegetation, landscape structure, climate, and occurrence and density of hosts (Dobson et al. 2011; Li et al. 2012; Gilbert et al. 2014; Vanwambeke et al. 2016; Boehnke et al. 2017; Requena-García et al. 2017; Jung Kjær et al. 2019). The natural habitat of *I. ricinus* are forests that provide a favorable environment for host search and off-host survival during the non-parasitic phase (Barandika et al. 2006; Tack et al. 2012b; Ehrmann et al. 2018). Especially, broadleaf and mixed forests with oaks (*Quercus* spp.), rich undergrowth and high density of hosts such as red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) are considered an ideal habitat for the occurrence of *I. ricinus* (Gilbert et al. 2012; Tack et al. 2012a; Hofmeester et al. 2017). Less favourable for *I. ricinus* are open habitats and young coniferous forests (Estrada-Peña 2001). Differences in tick densities may result from greater shrub cover which ensures more favourable microclimatic conditions for tick survival and influences the presence of hosts (Tack et al. 2012a). Another important factor influencing the abundance of ticks are the structural properties of the habitat and the proportion of forests, as confirmed by research conducted as part of the small FOREST project in southern and northern France, Belgium, western and eastern Germany, southern and central Sweden and Estonia (Ehrmann et al. 2017, 2018).

To assess the spatial distribution of tick occurrence and estimation of the risk of tick-borne disease, land cover maps are increasingly used (Ogden et al. 2008; Pepin et al. 2012; Braks et al. 2016; Garcia-Marti et al. 2017, 2018). The large-scale maps are useful for national risk assessment. However, the assessment of tick-borne risk on a local scale requires more detailed land cover characteristics and comprehensive understanding of the key drivers of tick abundance. In Poland, detailed land cover maps have been developed and made available by State Forests (https://www.bdl.lasy.gov.pl/portal/mapy) that take into account the diversity of forest habitat types. The map contains a division into 36 types of forest habitats distinguished on the basis of soil fertility and moisture content, similar climate features as well as its geological shape and structure.

The aim of this study was to estimate the tick-borne pathogen risk at the local scale in Lower Silesia, SW Poland, using detailed forest habitat-type maps. Regular 2-year field studies in the three most frequently recorded forest habitat types in Poland were conducted to determine: (1) tick abundance in the particular forest habitat type, (2) the prevalence of *I. ricinus*-infection with spirochetes and (3) influence of air temperature and humidity on the activity of ticks.

Material and methods

Study area

Field collections were performed in three forest habitat types distinguished according to the classification in force in Poland: broadleaf forest, mixed broadleaf and coniferous forest and coniferous forest. The forest habitat type was determined on the basis of land cover maps available in the Forest Data Bank (https://www.bdl.lasy.gov.pl/portal/mapy). In total, the research involved nine sites (3 sites in each forest habitat type; sites 1, 6, and 9 in broadleaf forest, sites 2, 5, and 7 in mixed broadleaf and coniferous forest, and sites 3, 4, and 8 in coniferous forest) located in the Miękinia Forest District of Lower Silesia, SW Poland (Fig. 1). All the designated sites were located within the forest complex to avoid ecotone influences and in some cases were located very close to each other, only a few hundred meters apart.

The selected forest habitat types constitute the largest area share of habitat types of forests in Poland (www.bdl.lasy.gov.pl). They cover lowland habitat types characterised by fresh moisture group of the habitat, which is, however, a varied species composition of dominant and admixed layer of trees, undergrowth and groundcover (Table 1; Fig. 2a–c) (https://www.lasy.gov.pl).

The diversity in terms of habitats in the Miękinia Forest District translates into a large species diversity of mammals, including European badger (*Meles meles*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), European rabbit (*Oryctolagus cuniculus*), beech marten (*Martes foina*), European pine marten (*Martes martes*), red fox (*Vulpes vulpes*), roe deer (*Capreolus capreolus*), European hare (*Lepus europaeus*), and red squirrel (*Sciurus vulgaris*) (https://www.lasy.gov.pl).

Tick collection

Host-seeking ticks were collected using the standard flagging method during their spring peak activity i.e. from April to June 2018 and from April to June 2019. At each of the

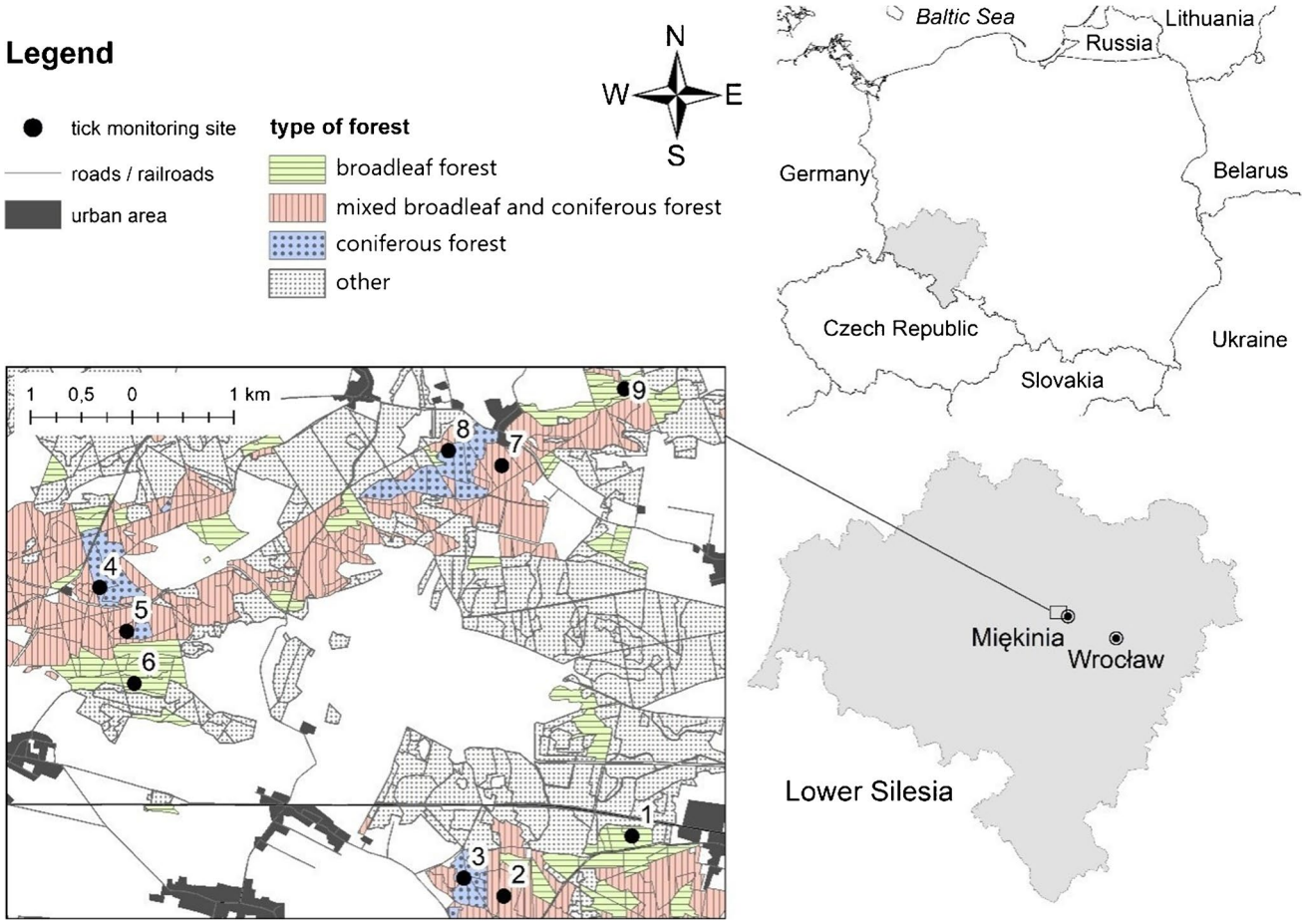

Fig. 1 Location of the nine study sites in three types of forest habitat on the area of the Miękinia Forest District, Lower Silesia, SW Poland

nine sampling sites, we measured 20 × 20 m quadrat via a tape measure. Within each quadrat, we delineated four sampling plots at intervals of 10 m, for a total of 4 plots per quadrat, each of 100 m^2. Samples from designated positions were randomly taken to eliminate time error, and sampling was carried out on dry and windless days between 9:00 and 15:00. After dragging for 1 min, the flag was thoroughly checked for ticks, and the caught ticks were removed with tweezers and were kept alive in containers with green leaf to maintain proper humidity. Then ticks were transported to the laboratory, where their species affiliation was determined, they were counted and tested for spirochete infection. The identification of the collected ticks was carried using a key for species identification under a stereomicroscope (Estrada-Peña et al. 2017). Only nymphs and adults of *I. ricinus* were included in the estimation of the abundance of ticks. The larvae were not included in the study because of their clustering occurrence in small areas. Only ticks identified as *I. ricinus* were studied for the presence of spirochetes.

In addition, during tick collection, the temperature and relative humidity were measured 1 m above ground level using a hygrometer (HANNA H19565). Each tick species was counted and its density of occurrence determined i.e. numbers per 100 m^2.

Identification of Borrelia infections

Nested PCR

DNA isolation was carried out using the ammonia method (Guy and Stanek 1991; Rijpkema et al. 1996). Test specimens of ticks were placed individually into Eppendorf tubes, and crushed in a 0.7-molar solution of ammonium hydroxide (NH_4OH). The lysates were stored at − 20 °C. To detect *Borrelia* spp., a nested PCR targeting the *fla* gene was used (Wodecka et al. 2009). The first reaction used primers 132f (5′-TGGTATGGGAGTTTCTGG-3″) and 905r (5′-TCT GTCATTGTAGCATCTTT-3″), which generated a product of 774 bp length. The second reaction used primers 220f (5′-CAGACAACAGAGGAAAT-3″) and 824r (5′-TCAAGT CTATTTTGGAAAGCACC-3″) resulting a product with a length of 605 bp. The reaction mixture for a single sample

Table 1 Species composition of the dominant and admixed layer of trees, undergrowth and groundcover in three selected forest habitat types (https://www.lasy.gov.pl)

Forest habitat type	Tree species		Undergrowth	Groundcover
	Dominant	Admixed		
Broadleaf forest	Sessile oak (*Quercus petrae* L.), scots pine (*Pinus sylvestris* L.)	Silver birch (*Betula pendula* Roth), European aspen (*Populus tremula* L.)	Common hazel (*Corylus avellana* L.), alder buckthorn (*Frangula alnus* Mill.)	Wood sorrel (*Oxalis acetosella*), lily of the valley (*Convallaria majalis*), western oakfern (*Gymnocarpium dryopteris* L.), wall lettuce (*Mycelis muralis*)
Mixed broadleaf and coniferous forest	Scots pine (*Pinus sylvestris* L.)	Silver birch (*Betula pendula* Roth), Norway spruce (*Picea abies* L.), European silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.), alder buckthorn (*Frangula alnus* Mill.), rowan (*Sorbus aucuparia* L.)	Rowan (*Sorbus aucuparia* L.)	Lily of the valley (*Convallaria majalis*), wild strawberry (*Fragaria vesca*), stone bramble (*Rubus saxatilis*)
Coniferous forest	Scots pine (*Pinus sylvestris* L.)	Silver birch (*Betula pendula* Roth)	Rowan (*Sorbus aucuparia* L.)	Schreber's big red stem moss (*Pleurosium schreberi*), leucobryum moss (*Leucobryum glaucum*), whortleberry (*Vaccinium myrtillus* L.), sheep's fescue (*Festuca ovina*)

had a volume of 25 µl: 12.5 µl 2×PCR Mix Plus (A&A Biotechnology), 2.5 µl of each primer, 4.5-µl sterile nuclease-free water and 3 µl of template DNA for the first reaction, and 12.5 µl-2×PCR Mix Plus (A&A Biotechnology), 2.5 µl of each primer, 5.5 µl-sterile nuclease-free water and 2 µl of the outer PCR product for nested PCR. PCR reactions included an initial denaturation at 95 °C for 3 min, 35 cycles each consisting of denaturation at 95 °C for 45 s, attachment of primers at 50 °C (132f and 905r) or at 54 °C (220f and 824r) for 45 s, elongation of the DNA chain 72 °C for 1 min and a final elongation step at 72 °C for 5 min.

The separation of nested PCR products was carried out by electrophoresis on a 1.5% agarose gel with the addition of a nucleic acid stain SimplySafe (Eurx) against DNA mass standards (Marker 1 100–1000 bp A&A Biotechnology). The separation of products was carried out at 100 V for 30 min on the Cleaver Scientific CS-300 V omniPAC MIDI Power Supply apparatus. The results of the PCR were viewed under UV light and were archived in computer storage using Quantity One Basic Software (Bio-Rad). A product of a size of 605 bp was considered positive.

To check the presence of spirochetes, 494 randomly selected ticks of *I. ricinus* (max. of 30 ticks from each site in that year — the exceptions were sites 4, 7, and 10 of which all collected ticks were tested) including 374 nymphs, 60 females and 60 males were tested, which constituted only a subset of all collected ticks.

PCR–RFLP

To determine the individual species in a complex of *B. burgdorferi* s.l., PCR–RFLP (PCR-restriction fragment length polymorphism) analysis was used (Wodecka 2011). Digestion with HpyF3I generates patterns and allows the detection of species included in the complex *B. burgdorferi* s.l. (*B. afzelii*, *B. garinii*/*B. bavariensis*, *B. valaisiana*, *B. bissetti*, *B. lusitaniae*, *B. spielmanii*, *B. burgdorferi* sensu stricto) and *B. miyamotoi*. Positive nested PCR products obtained using primers 220f and 824r were digested with the restriction enzyme HpyF3I (ThermoFisher Scientific, USA) which recognises CTNAG sequences. The digestion of PCR products was carried out as described in the Thermo Scientific FastDigest HpyF3I protocol. The digestion products of restriction enzyme HpyF3I were analysed on a 3% agarose gel and archived as described above. To confirm species identification, selected nested PCR products were subjected to purification using a DNA purification kit (GenoPlast Biochemicals), following the instructions provided by the manufacturer and then sent for sequencing (Genomed, Poland). Sequencing was carried out for all samples that resulted in similar restriction patterns (e.g. *B. garinii* and *B. bavariensis)*, as they could not be distinguished by

Fig. 2 Designated habitats for the tick collection: (a) broadleaf forest, (b) mixed broadleaf and coniferous forest, (c) coniferous forest

the used PCR–RFLP method. The obtained sequences were compared with nucleotide sequences deposited in the GenBank database at the National Center for Biotechnology Information (NCBI), using the BLAST (Basic Alignment Search Tool) program.

Statistical analysis

In order to determine possible relationships between tick abundance of *I. ricinus* and selected factors such as a month, forest habitat type, air temperature, and relative humidity, we decided to use GLMM (generalized linear mixed model–negative binomial regression) approach. This methodology is widely used especially in problems where a response variable is discrete or non-normally distributed in general. The same approach was used to the detect influence of the type of forest on *Borrelia* spp. prevalence (binominal regression) (Bolker et al. 2009). All the statistical analyses were performed in R.

Results

In the period from April to June 2018 and 2019, at nine designated sites a total of 2196 (10.1/100 m^2) ticks were collected, including 2093 *Ixodes ricinus* (95.3%; 9.6/100 m^2), 46 *Dermacentor reticulatus* (2.1%; 0.2/100 m^2) and 57 *Haemaphysalis concinna* (2.6%; 0.3/100 m^2). From the collected *I. ricinus*, 589 were larvae (28.1%; 2.7/100 m^2), 1261 nymphs (60.3%; 5.8/100 m^2), 128 females (6.1%; 0.6/100 m^2) and 115 males (5.5%; 0.5/ 100 m^2). The number of *I. ricinus* ticks in individual sites varied from 20.9/100 m^2 specimens at site 9, to 0.6/100 m^2 specimens at site 8 (Table 2). There were significant differences in the observed density of *I. ricinus* between months: higher densities were recorded in May and June compared to April (Fig. 3).

A varied abundance of *I. ricinus* was also noted between forest habitat types. The highest number of ticks were collected in broadleaf forests (15.0/100 m^2), then in mixed broadleaf and coniferous forest (3.8/100 m^2) while the lowest number in coniferous forests (2.0/100 m^2) (Table 3). In

Table 2 Mean density of *I. ricinus* ticks (excluding larvae) per 100 m^2 collected in nine sites of the Miękinia Forest District, Lower Silesia, SW Poland in 2018 and 2019

Developmental stage of ticks	Study sites									Total
	1 BF	2 MBCF	3 CF	4 CF	5 MBCF	6 BF	7 MBCF	8 CF	9 BF	
Nymphs	14.7	5.9	1.6	0.9	2.4	6.2	0.9	0.4	19.4	5.8
Females	1.5	0.5	0.7	0.8	0.7	0.4	0.1	0.1	0.6	0.6
Males	1.1	0.4	0.8	0.5	0.4	0.4	0.1	0.1	0.9	0.5
Total	17.3	6.8	3.1	2.2	3.5	7.0	1.1	0.6	20.9	6.9

**BF* broadleaf forest, *MBCF* mixed broadleaf and coniferous forest, *CF* coniferous forest

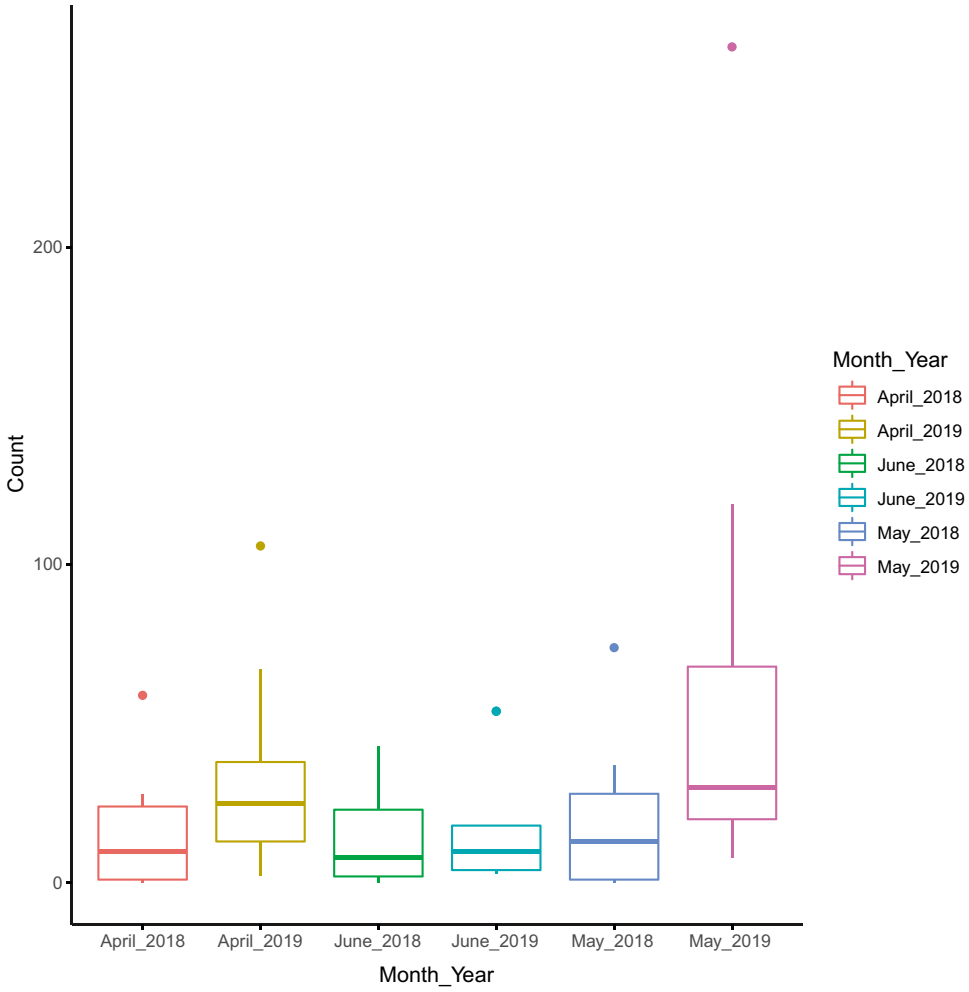

Fig. 3 Box plots of monthly differences in tick abundances (y-axis) collected for selected months i.e. April, May, June in 2 years 2018 and 2019 (x-axis). The thick horizontal line indicates the median (IQR, the inter-quartile range), the box shows the range between the first and the third quartiles, and the whiskers determine the range without outliers for the assumption 1.5 × IQR. It is easy to see the highest difference it observed between May 2018 and 2019, as well as April 2018 and 2019

Table 3 Mean density of *I. ricinus* ticks (excluding larvae) per 100 m^2 collected on different forest habitat types

Forest habitat types*	No. of collected ticks	Developmental stage of ticks		
		Nymphs	Females	Males
BF	15.0	13.4	0.8	0.8
MBCF	3.8	3.1	0.4	0.3
CF	2.0	1.0	0.5	0.5
Total	6.9	5.8	0.6	0.5

**BF* broadleaf forest, *MBCF* mixed broadleaf and coniferous forest, *CF* coniferous forest

order to determine possible relationships between abundance of *I. ricinus* and selected factors such as month, forest habitat type, air temperature and relative humidity, the GLMM was used assuming a negative binomial distribution for the response variable i.e. tick abundance and log link. In this model, we used raw data without any transformations, but we excluded information about the year of data collection. Moreover, we included the site variables as a random factor. Furthermore, we disregarded the humidity variable due to its high linear correlation with temperature ($r = -0.749557529816691$) (Fig. 4), which makes our model easier to interpret. As a result, we could present the influence of studied factors on the response variable simultaneously. In particular, we got that such factors as: May (coefficient = 1.34548, p-value = 5.76e − 12), June (coefficient = 1.35246, p-value = 3.47e − 06), broadleaf forest (coefficient = 1.87267, p-value = 2.79e − 07) and mixed broadleaf and coniferous forest (coefficient = 0.34878, p-value = 0.277) have positive influence on the tick abundance, though mixed broadleaf and coniferous forest coefficient is not statistically significant. This is in contrast to the temperature coefficient (coefficient = − 0.21559, **p**-value = 1.08e − 12) which has a negative impact on the response variable. The detailed computations with model validations can be found in the supplementary material.

During tick collection, the ambient air temperature ranged from 14.7 to 27.6 °C in 2018 and from 10.8 to 25.8 °C in 2019, and the relative humidity ranged from 23.3 to 56.3% in 2018 and 44.1 to 72.6% in 2019. In

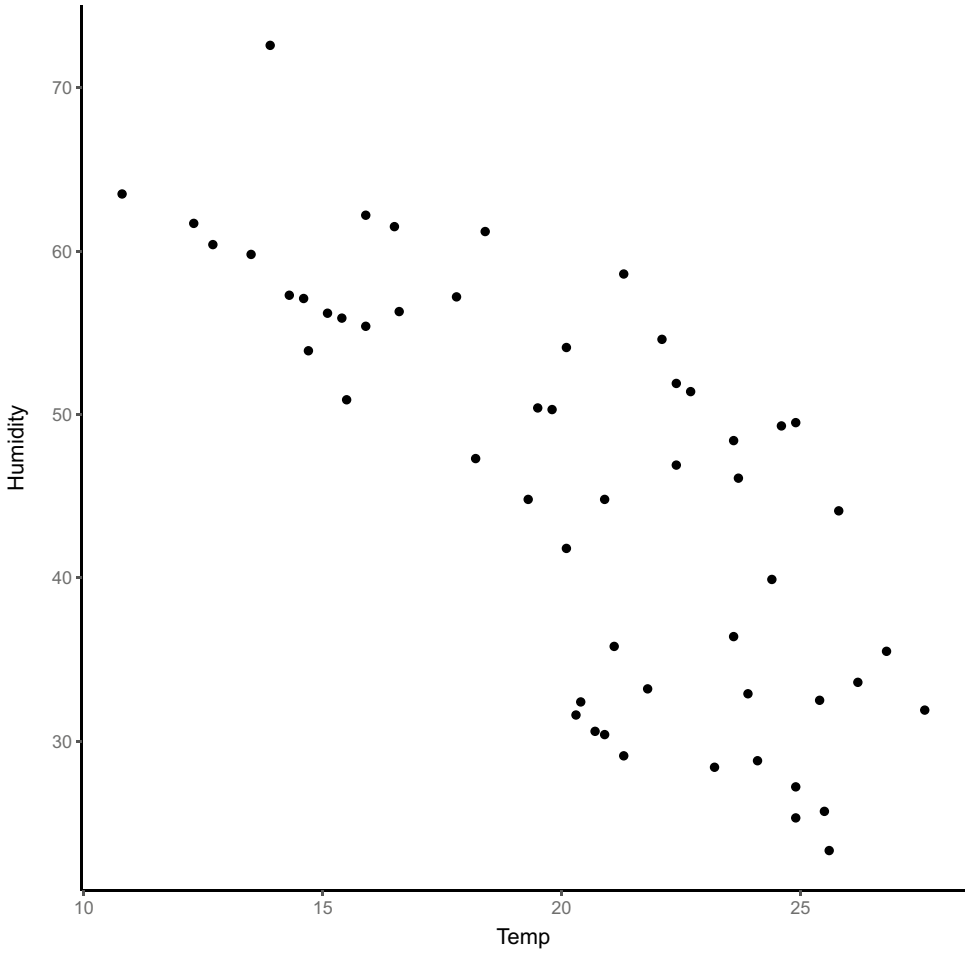

Fig. 4 The relationship between temperature (x-axis) and humidity (y-axis). The shape of this plot suggests linear dependency between these variables. This observation is supported by relatively high level of correlation coefficient ($r = -0.749557529816691$)

addition to a significant effect of temperature at 1 m above ground level with a higher density of ticks being recorded at lower temperatures, we also observed the positive influence of the relative humidity with a higher density of ticks being recorded at higher humidity.

The overall prevalence of *Borrelia* spp. infection was 16.8%. PCR–RFLP gave restriction patterns characteristic of different *Borrelia* species. Four genospecies from the *B. burgdorferi* s.l. complex were identified: *B. afzelii* (30.1%), *B. garinii* (38.6%), *B. valaisiana* (2.4%), *B. lusitaniae* (18.1%). In addition, *B. miyamotoi* (9.6%) and co-infection with *B. miyamotoi*/*B. lusitaniae* (1.2%) were found (Table 4). The influence of the forest habitat type on the level of infection with *Borrelia* spp. was analysed only for nymphs using GLMM with binomial structure. We decided not to take into account "site" as a random factor due to the small data size. As a result, we found that mixed broadleaf and coniferous forest (coefficient = 1.2626, p-value = 0.0261) has a significant positive influence. The detailed computations with model validations can be found in supplementary materials.

Discussion

Identification of environmental factors influencing the abundance of ticks and the frequency of *Borrelia burgdorferi* s.l. is essential for assessing the risk of tick-borne diseases in the environment, both on a large and local scale. For risk assessment, the land cover maps are increasingly used as a source of environmental data. For the purposes of estimation of the tick abundance on a local scale we used in our study, the land cover maps available from the Forest Data Bank. The used maps present forest habitat types according to the classification system in Poland. The forest habitat type, as the basic unit in the forest habitat classification, includes forest areas with similar habitat conditions resulting from soil fertility and moisture, similar climate features, terrain topography and its geological structure (Kliczkowska 2006). Our study covered three types of forest habitat: broadleaf forest, mixed broadleaf and coniferous forest and coniferous forest, which account for 50.8% of the forest area in Poland (http://www.lasy.gov.pl).

Table 4 Results of genotyping of *Borrelia* spp. isolated from *I. ricinus* according to developmental stage and forest habitat

	No. of		No. of ticks positive for genospecies of *Borrelia* spp. (%)					
	Tested ticks	Infected ticks (%)	*B. afzelii*	*B. garinii*	*B. valaisiana*	*B. lusitaniae*	*B. miyamotoi*	Co-infection*
Stage								
Nymphs	374	51 (13.6)	20	23	0	3	4	1
females	60	19 (31.6)	3	7	1	5	3	0
males	60	13 (21.6)	2	2	1	7	1	0
Total	494	83 (16.8)	25 (30.1)	32 (38.6)	2 (2.4)	15 (18.1)	8 (9.6)	1 (1.2)
Forest types**								
BF	180	26 (14.4)	14	11	0	0	1	0
MBCF	122	21 (17.2)	4	11	0	3	3	0
CF	72	4 (5.6)	2	1	0	0	0	1
Total	374	51 (13.6)	20 (39.2)	23 (45.1)	0 (0.0)	3 (5.9)	4 (7.8)	1 (2.0)

* Coinfection: *B.miyamotoi/B.lusitaniae*, ** the number of ticks tested includes only nymphs (*BF broadleaf forest, MBCF mixed broadleaf and coniferous forest, CF coniferous forest*)

The dominant collected tick species in all types of forest habitat was *Ixodes ricinus*, while *Dermacentor reticulatus* and *Haemaphysalis concinna* were less frequent. The occurrence of these three tick species in Lower Silesia was confirmed by previous studies by Karbowiak and Kiewra (2010), Kiewra (2014) and Kiewra et al. (2019). The results of the latter studies also showed a predominance of *I. ricinus* in the population of collected tick species.

In our study, the highest density of ticks was recorded in May and June compared to April. Similar peak activity was observed in SW Poland (Kiewra 2014), in eastern Poland (Zając et al. 2021) and in southern Germany (Schulz et al. 2011), whereas in Finland (Sormunen et al. 2020) the observed peak activity was longer i.e. from May to September.

The type of forest habitat according to the classification system in Poland turned out to have a significant impact on the occurrence of *I. ricinus*, suggesting that the detailed forest habitat-type maps are useful for tick-borne risk assessment. The highest number of *I. ricinus* was collected in broadleaf forest with dominant oak stands (15.0/100 m^2), while in mixed broadleaf and coniferous forest, and coniferous forest, the abundance of *I. ricinus* was much lower accounting for 3.8/100 m^2 and 2.0/100 m^2, respectively. The significance of the influence of forest type on the number of ticks has been also reported by studies conducted in other countries. Higher numbers of *I. ricinus* ticks in oak stands compared to pine stands were found in northern Spain and in northern Belgium (Estrada-Peña 2001; Tack et al. 2012a; Ruyts et al. 2018). However, it should be noted that the presence and density of ticks depend not only on the type of forest, but also on the richness of the layer of low vegetation and undergrowth, which affects humidity and temperature in an environment, and increases the access to potential hosts (Perez et al. 2016). Research confirming this fact was carried out, among others, in the Czech Republic and Belgium, where a lower density of ticks was recorded in forests in which shrub and undergrowth were removed compared to forests in which such treatment was not carried out (Hubálek et al. 2006; Tack et al. 2013).

In addition, the microclimate of the habitat has a significant impact on the occurrence of ticks in the environment. It needs to be highlighted that microclimatic data measured directly in the forest can differ from conditions recorded by weather stations (Boehnke et al. 2017).

In our study, during collecting ticks, the ambient air temperature measured at the time of tick collection ranged from 10.8 to 27.6 °C, and the relative humidity ranged from 23.3 to 72.6%. In a wide range of humidity and temperature, active ticks of *I. ricinus* were collected also in Germany (Gethmann et al. 2020), Belgium (Tack et al. 2012b) and in a previous study in Lower Silesia (Kiewra 2014). Furthermore, the relative humidity and temperature are key factors influencing tick activity. The influence of air temperature is often considered together with humidity, as these two factors in the environment are closely related to each other and regulate the life cycles of ticks. In our study, a significant influence of air temperature and relative humidity on the abundance of ticks was observed. Furthermore, we observed a high linear correlation between relative humidity and air temperature ($r = -0.749557529816691$). Therefore, the unique microclimate at the designated sites and the changes in temperature and humidity that occur in them may affect the number of ticks. In Norway, Andreassen et al. (2012) found that relative humidity and temperature had an impact on the TBEV (tick-borne encephalitis virus) prevalence in ticks of *I. ricinus*. Therefore, when estimating the prevalence of tick-borne pathogens in *I. ricinus*, in situ measurements must be carried out to obtain good estimates of the complex temperature and relative humidity conditions in forests.

Deciduous forests represent a habitat characterized by higher densities of small rodents and roe deer compared to pine forests and thus provide a greater possibility of feeding for ticks (Tack et al. 2012a). However, the presence of reservoir hosts in the habitat affects not only tick abundance, but also the prevalence of *B. burgdorferi* s.l. In our study, the overall mean prevalence of infection in host-seeking ticks was 16.8%, which is in line with the average infection rate in Europe (Strnad et al. 2017). In addition, in previous studies conducted in forested areas of Lower Silesia (Park Osobowicki, Ślężański Landscape Park), the minimum prevalence of infection of *I. ricinus* with *Borrelia* spirochetes was likewise estimated by 15.5% (Kiewra et al. 2014). A positive effect in the infection level of *Borrelia* spp. was found for broadleaf forest and mixed broadleaf and coniferous forest; however, this effect was only significant for mixed broadleaf and coniferous forest. A similar influence of forest habitat types on the prevalence of *I. ricinus* infection was found in the Lublin province (Wójcik-Fatla et al. 2016).

Genotyping by PCR–RFLP revealed the presence of five species including four belonging to the *Borrelia burgdorferi* s.l. complex: *B. garinii* (38.6%), *B. afzelii* (30.1%), *B. valaisiana* (2.4%) and *B. lusitaniae* (18.1%), as well as *B. miyamotoi* (9.6%) — the species causing relapsing fever. Earlier studies in Lower Silesia revealed the presence of *B. burgdoreri* s.s. and a relatively high prevalence of *B. lusitaniae* and *B. miyamotoi* (Kiewra 2014; Kiewra et al. 2014). The absence of *B. burgdorferi* s.s. in our study corresponds with a low prevalence of *B. burgdorferi* s.s. in *I. ricinus* in Lower Silesia, as reported in previous studies. The predominant presence of *B. afzelii* and *B. garinii* is in line with other studies. Kowalec et al. (2017) observed in natural, endemic areas of north-east (NE) Poland (Białowieża) and urban areas of central Poland (Warsaw) a predominance *B. afzelii* (69.3%) in urban and *B. garinii* (48.1%) in natural areas. Furthermore, a predominance of *B. afzelii* and *B. garinii* was also found in studies reviewing the existing data published in Europe in the period 2010–2016 on the frequency of *B. burgdorferi* s.l. spirochetes in *I. ricinus* ticks (Strnad et al. 2017). *B. afzelii* is most often acquired by tick larvae and nymphs that parasitise-infected rodents, while *B. garinii* is associated with birds. Both these tick hosts have been documented as tick reservoirs (Takumi et al. 2019). Our results demonstrate that predominantly *I. ricinus* nymphs acquire an infection of *B. afzelii* (39.2%) and *B. garinii* (45.1%) compared to adult ticks (*B. afzelii* 15.6% and for *B. garinii* 28.1%).

In conclusion, vegetation which provides protection against adverse environmental conditions and access to hosts is essential for the survival and development of *I. ricinus*, and their numbers may vary in different habitat types. Forests with predominantly deciduous stands with a well-developed layer of shrubs and litter provide a cooler, more humid microclimate where ticks can find shelter and where potential hosts hide. A comprehensive statement of factors determining a specific forest habitat type, as well as available maps on forest resources and forest conditions, can be the basis for determining the spatial distribution of ticks. In our study, broadleaf forest and mixed broadleaf and coniferous forest turned out to be areas of higher tick-borne risk than coniferous forests. The land cover maps used in our own study can be useful at the local scale for the estimation of areas with the highest tick-borne risk.

Given the fact that in our research the study sites were at a slight distance from each other, allowing only limited conclusions regarding larger spatial scales, similar studies should be conducted with sampling areas that are spread over larger spatial scales, to confirm the results.

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Declarations

Conflict of interest The authors declare no competing interests.

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