

Prevalence of *Borrelia burgdorferi* in ticks removed from skin of people and circumstances of being bitten – research from the area of Poland, 2012–2014

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Abstract

Introduction. During feeding, the tick sucks blood from the host along with the pathogens that are in the blood, simultaneously exchanging its own pathogens with the host. Humans can also be a host. It is important to understand the most typical circumstances in which people might become infected with *Borrelia burgdorferi*. This knowledge will help to prepare health education programmes aimed at the prevention of Lyme disease and other tick-borne diseases.

Objective. The aim of the study was to determine the percentage of ticks infected with *Borrelia burgdorferi* sensu lato, depending on the circumstances of getting bitten.

Materials and method. The research material consisted of ticks acquired from people who had been bitten, and questionnaires completed by these people. 510 ticks were acquired from 257 females and 253 males. Following delivery of a tick for testing, the stage of its development was determined and a molecular assay of *Borrelia burgdorferi* DNA performed.

Results. A positive result of the nested-PCR test was obtained in 78 ticks, which represents 15.30% of all ticks. The infected ticks were collected from male (41 ticks – 52.56%) and female subjects (37 ticks – 47.44%). The biggest number of infected ticks were collected in autumn (54 ticks – 69.23%) and from people who had been into forests (44 ticks – 56.41%). Among the people from whom the infected ticks were acquired, the dominating group included persons over 16 years of age (53 persons – 67.95%) and children aged 0–5 years (16 persons – 20.51%). One in four infected ticks were acquired from the southwestern (20 ticks – 25.64%) and eastern regions of Poland (21 ticks – 26.92%).

Conclusion. Infestation of ticks infected with Lyme disease spirochete in this study proved to be variable and depend on the season, the area of tick attack and the region in Poland. The results of the study clearly show that ticks infected with *Borrelia burgdorferi* inhabit all regions of Poland. The results are consistent with National Institute of Hygiene data which indicates that Lyme disease cases are recorded in all regions of Poland.

Key words

ticks, *Borrelia burgdorferi*, tick-borne disease, Lyme disease, tick's bite

INTRODUCTION

Ticks are arachnids that live in warm and humid places which include areas overgrown with vegetation, particularly with tall grasses and shrubs. They dwell primarily in deciduous and mixed forests, as well as on the borders of forests and fields. This is the natural habitat of these arachnids. In recent years, increased populations of ticks have been observed in urban areas, as well as in orchards, parks and gardens [1–3]. This is caused primarily by human activities, on the one hand, due to the prohibition of burning of agricultural wastelands

that has been in effect for a number of years, on the other hand, it is due to the more frequent human interaction with forest areas (i.e. through tourism and recreation), which enables ticks to move from their natural environment to urban areas and human settlements [4, 5].

Ticks have a complex life cycle, they develop from larva, through nymph, and into the adult form called the imago. To move to the next stage of development, the tick must feed on vertebrate blood. During the feeding, the tick sucks the blood of the host along with the pathogens that are in the blood, while at the same time exchanging its own pathogens with the host. As indicated by scientific research, this enables the ticks to carry bacteria (i.e. *Borrelia burgdorferi*, *Bartonella spp.*, *Coxiella burnetii*, *Rickettsia spp.*, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Leptospira spp.*), viruses (i.e. Tick-borne encephalitis virus, Crimean-Congo haemorrhagic

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fever) or protozoa (i.e. *Toxoplasma gondii*, *Babesia divergens*, *Babesia microti*) [6–14].

The tick-borne disease most frequently reported in case statistics, both in Poland and other countries in Europe, is Lyme disease [5, 15, 16]. Epidemiological analyses and scientific research show that Poland constitutes an endemic area for the disease, i.e. there are no places or areas where ticks are not infected with *Borrelia burgdorferi* [4]. The incidence of this disease is variable. A systematic increase in the number of new cases in Poland has been reported for years. According to the National Institute of Hygiene, the incidence in 2010 was 23.58/100,000 (9,005 cases), in 2011 – 23.88/100,000 (9,159 cases), in 2012 – 22.60/100,000 (8,786 cases), in 2013 – 33.12/100,000 (12,763 cases), and in 2014 – 36.1/100,000 (13,866 cases) [17]. Data from the neighbouring countries show higher rates of incidence – e.g. in Mecklenburg-Western Pomerania it was 74.8/100,000 in 2011 and in Brandenburg (Germany) – 60/100,000 in 2011 [18]. In the Czech Republic, the incidence in 2008 was 41.6/100,000, with most cases of Lyme disease diagnosed in children aged 5–9 years (66/100,000 in 2011) and in the group of people aged 50–74 years (62–71/100,000 in 2011), and this trend persists to this day [19]. Slightly different incidence rates have been reported for Slovakia – depending on the region; the incidence in this country ranges from 16.24–21.18/100,000 [20].

Borrelia afzelii and *Borrelia garinii* are dominant genospecies in ticks in Europe [21–29]. In North America there is one major genospecies – *Borrelia burgdorferi sensu stricto* [16]. These genospecies colonize different organs, due to which the symptoms of the disease may vary considerably in patients from both continents [30–32]. In Europe, Lyme disease most commonly affects the nervous system, whereas in North America joint symptoms prevail.

It is assumed that not every bite of a tick infected with *Borrelia burgdorferi* will lead to human infection. In order for the bacterium to survive the change of the host ability to multiply in the new body, it must alter some of its proteins, and – by extension – its antigens [33–36]. This process takes time, which is estimated at several hours [37]. Thus, quick removal of the tick reduces the risk of infection but it does not eliminate it completely. Therefore, it should be assumed that each contact of an infected tick with the host's blood is dangerous and may cause infection.

OBJECTIVE

The aim of this study was to determine the percentage of ticks infected with *Borrelia burgdorferi sensu lato* depending on the circumstances of getting bitten. It is important to understand the most typical circumstances in which people might become infected with *Borrelia burgdorferi*. This knowledge will help to prepare health education programmes aimed at the prevention of Lyme disease and other tick-borne diseases.

MATERIALS AND METHOD

The research material consisted of ticks acquired from people who had been bitten, and questionnaires completed by these people, or in the case of children – by their parents. The research was conducted throughout Poland during the spring and autumn of 2012, 2013 and 2014 and did not include

individuals with professions exposing them to frequent contact with ticks (i.e. foresters, farmers). 510 ticks and 510 questionnaires were collected. Ticks were filled with blood. Following delivery of a tick for testing, the stage of its development (larva, nymph, female, other: male, *Dermacentor reticulatus*) was determined and a molecular assay of *Borrelia burgdorferi DNA* was performed. The self-designed questionnaire included questions about the area where the bite may have presumably occurred (forest, meadow, garden, other, I don't know), as well as the gender and age of the bitten person, how the tick was removed from the skin (using domestic methods, in a health care facility), the number of bites (first, subsequent) and estimated duration of the tick's residence on the skin (up to 12 hours, 12–24 hours, more than 24 hours, I don't know). Based on the information regarding the area where the bite took place, the region of the tick's origin in Poland was determined – a division according to the Classification of Territorial Units for Statistics was used, also called the Nomenclature of Territorial Units for Statistics (French: *NUTS- Nomenclature des Unités territoriales statistiques*). This classification divides Poland into 6 regions, i.e. northern, northwestern, eastern, central, southwestern and southern regions.

The material was obtained in cooperation with the Polish Lyme Disease Foundation 'Bartek' which had advertised through social and traditional media the opportunity of having a free tick examination.

DNA was isolated from ticks by means of a GeneMATRIX set (*EURx*, Gdańsk Poland). Next, the isolated DNA was amplified using nested PCR. A fragment of the gene encoding flagellin (*fla*) was amplified using primers specific for gene sequences of flagellin for *Borrelia burgdorferi sensu lato* complex (*B. burgdorferi in the strict sense*, *B. garinii*, *B. afzelii*, *B. valaisia*, *B. miyamotoi*). The sequences are reserved *Blirt's DNA*, Gdańsk, Poland patent. The amplified product of 259 bp was detected after electrophoresis in 1% agarose gel. *Blirt DNA*, Gdańsk Poland procedure was used (*Borrelia diagnostic kit cat. PK17N*).

The results were analyzed statistically – Chi-square test and Fisher's test were used as needed. Statistical software was used (*StatSoft*, Krakow, Poland). The significance level was set at $p < 0.05$.

RESULTS

510 ticks were acquired from 257 females and 253 males. The great majority – i.e. 493 ticks (96.66%) – belonged to the *Ixodes ricinus* species; the *Dermacentor reticulatus* species accounted for 1.96% (10 ticks). 294 ticks were acquired during spring and 216 ticks were acquired during autumn. Ticks were collected primarily from persons over 16 years of age (288 people – 56.47%). Another large group consisted of children aged 0–5 years (119 people – 23.33% (Tab. 1).

Table 1. Gender and age of people bitten by ticks

Nested PCR test result	Gender		Age			
	Female	Male	0–5 years	6–10 years	11–15 years	16 years and over
Positive	37/253 (14.6%)	41/257 (15.9%)	16/119 (13.4%)	8/86 (9.30%)	1/17 (5.9%)	53/288 (18.4%)
P value	0,67679		0,11122			

Table 2. Nested PCR test results depending on the form of development of the tick, probable area of tick attack and the season

Nested PCR test result	Form of development of the tick				Probable area of tick attack ¹				Season		
	Larva	Nymph	Female	Other	Forest	Meadow	Garden	Other	I don't know	Spring	Autumn
Positive n=78 (15.30%)	4/22 (18.2%)	36/262 (13.7%)	33/203 (16.3%)	5/23 (21.8%)	44/234 (18.8%)	10/97 (10.3%)	22/158 (13.9%)	7/46 (15.2%)	4/40 (10%)	24/294 (8.2%)	54/216 (25%)
P value	0,70046	0,31637	0,62354	0,37951	0,04262*	0,12958	0,56467	0,98791	0,33253	0,00000*	

* statistically significant

¹ Results do not add up to 100% because respondents were allowed to indicate up to two answers.

More than half of the analyzed population (56.47%) stated that it was the first time they had been bitten by a tick. The predominant stage of tick development was the female (203 ticks – 39.80%) nymph (262 ticks – 51.37%). More than 75% of all ticks (392 ticks – 76.86%) were removed from the skin using domestic methods. When asked about the estimated time of residence of the tick in the skin, the person in question usually indicated a period of up to 12 hours (49.02%); one person in 10 indicated a period of more than 2 days (10.98%). The forest was mentioned as the most probable area of tick attack (234 ticks – 45.88%).

The biggest number of ticks were acquired from the southern (115 ticks – 22.55%), eastern (104 ticks – 20.39%) and central (113 ticks – 22.16%) regions of Poland, and the smallest number – from the northwestern region (33 ticks – 6.47%). The biggest number of larvae was acquired from people bitten in the northern (7 ticks – 31.82%) and southwestern regions (6 ticks – 27.27%). Not a single larva specimen was acquired from the northwestern region. By contrast, nymphs were collected primarily in the southern region (74 ticks – 28.24% and the smallest number of nymphs collected from the northwestern region (14 ticks – 5.34%). In turn, female specimens were acquired primarily from the central (58 ticks, 28.57%) and eastern regions (46 ticks – 22.66%). The smallest number of female specimens was acquired from the northwestern region (19 ticks – 9.36%). These results proved to be statistically significant ($p < 0.05$).

A positive result of the nested-PCR test was obtained in 78 ticks, which represents 15.30% of all ticks. The infected ticks were collected from male (41 ticks – 52.56%) and female subjects (37 ticks – 47.44%). The biggest number of infected ticks was collected in autumn (54 ticks – 69.23%) and from people who had been going into forests (44 ticks – 56.41%). These results proved to be statistically significant. The female (33 ticks – 42.31%) and nymph (36 ticks – 46.15%) was the dominating form among the infected ticks (Tab. 2).

Among the people from whom the infected ticks were acquired, the dominating group included persons over 16 years of age (53 persons, i.e. 67.95%) and children aged 0–5 years (16 persons, i.e. 20.51%). However, these results did not prove to be statistically significant.

A statistically significant relationship was established between the result of the nested-PCR test and the region of Poland where ticks were acquired ($p < 0.05$). The analysis shows that one tick in 4 in the southwestern region (20 ticks from 75, i.e. 26.67%) and one tick in 5 in the eastern region (21 ticks from 104, i.e. 20.19%) were infected with *Borrelia burgdorferi*. In turn, the southern and central regions were characterized by the lowest percentage of the infected ticks acquired (8.70% and 8.85% respectively) (Tab. 3).

Table 3. Nested PCR test results depending on region of origin of the tick

Region of Poland	Positive
Northwestern	6/33 (18.2%)
Northern	11/70 (15.7%)
Eastern	21/104 (20.2%)
Central	10/113 (8.8%)
Southwestern	20/75 (26.7%)
Southern	10/115 (8.7%)
Total	78/510 (15.3%)
P value	0.00427*

*statistically significant

DISCUSSION

The presence of genetic material of the *Borrelia burgdorferi* spirochete in ticks may indicate a Lyme disease risk among people residing in the area where the ticks were collected. Most of the previously conducted research concerned ticks collected from relatively small areas, such as forests, urban parks and meadows, using the flagging method. The research on ticks obtained in such a way indicates that ticks infected with the *Borrelia burgdorferi* spirochete dwell both in areas traditionally associated with these arachnids (forests), as well as in areas located near human dwellings (kitchen gardens, parks). However, there is relatively little research regarding ticks obtained from bitten human hosts. The results of the current study focus on ticks removed from the skin of people they were feeding on.

Borrelia burgdorferi was detected in 78 out of 510 ticks, which accounts for 15.30%. These results differ from the results obtained by other researchers. In a study conducted in 2010 in Romania, the percentage of infected ticks amounted to 11.1%; however, it must be pointed out that the study was performed during spring and summer and focused on ticks removed from people within a single region [38] 518 were *Ixodes ricinus*, 10 *Dermacentor marginatus*, and 3 *Haemaphysalis* spp. ticks, and one unidentified tick due to destruction. Since evaluation of the hbb PCR revealed that it was not possible to differentiate between *B. spielmanii*/. *B. valaisiana* and *B. garinii*/. *B. bavariensis*, sequencing of an 800-bp fragment of the ospA gene was performed in these cases. Out of 389 investigated ticks, 43 were positive by hbb PCR for *B. burgdorferi sensu lato*. The positive samples were 42 *Ixodes ricinus* (11.1%) *B. burgdorferi sensu lato* prevalence. In turn, a German study conducted in 2009, focusing on ticks removed from the skin of soldiers residing in military training grounds, indicated that the infestation of ticks was 5.3% [39]. This study was conducted throughout the entire year. By contrast, in a study conducted in an endemic area in Switzerland between 2003 and 2005, the

percentage of infected ticks was 32.8% [40]. According to research conducted between 2004 and 2008 in a small island village in the Netherlands, 16% of ticks were infected [41]. Results similar to those of the Romanian study were obtained from research conducted in 1998 in 2 regions in Germany, yielding an average percentage of infected ticks of 11.3% [42]. In turn, Italian research on ticks acquired between 1995 and 2011 from people who were bitten in 4 regions in Italy have shown a small percentage of infected ticks – 0.3–0.5% [43] which cause effects in animals and humans, commonly referred to as tick-borne diseases (TBDs).

The most prevalent forms of removed ticks were nymph – 262 ticks (51.37%) and female – 203 ticks (39.8%); the larva accounted for 4.32% of acquired ticks (22 ticks). In the German study, the majority of ticks removed from the skin of soldiers stationed on the military training grounds were also nymphs (63.9%); the proportion of larvae was 24.7% and the adult females accounted for 10.9% of all ticks [39]. Nymphs were also prevalent in the Swiss study (64%), with significantly fewer females (33.3%) and larvae (1.6%) [40]. By contrast, the breakdown of the Dutch research was 56% of nymphs, 28% of females and 16% of the larvae [41]. Nymphs also prevailed in the Italian research (41%), with less females (34.6%) and the least larvae (8.4%) [43] which cause effects in animals and humans, commonly referred to as tick-borne diseases (TBDs).

In the presented study, among the infected ticks (78 ticks, i.e. 15.30%) nymphs accounted for 46.15% (36 ticks), females for 42.31% (33 ticks) and larvae for 5.13% (4 ticks). The remaining ticks, i.e. one male, 2 *Dermacentor reticulatus* males and 2 other males, comprised 6.41%.

The *Borrelia burgdorferi* DNA was detected in 18.18% of larvae, 13.74% of nymphs and 16.25% of females. In the German research of ticks acquired from soldiers, the corresponding results were as follows: 3.5%, 4.4% and 13% [39]. By contrast, in the Romanian study the infected ticks accounted for 10.7% of nymphs and 12.6% of adult specimens. In turn, infected ticks in the Dutch study included primarily the adult forms (20%), followed by nymphs (approx. 16%), whilst the percentage of infected larvae accounted for approx. 2% [41]. These studies indicate considerable differences in the individual forms of development of infected ticks, with a predominance of the adult form and a minor proportion of specimens infected in the larval stage. The issue that requires further research is the fact that the percentage of infected larvae in the current study was as high as 18.18%. This is a most striking phenomenon, as transovarial transmission of the infection, i.e. from an infected female to the larva of the *I. ricinus* ticks, was essentially not observed, while the presence of spirochetes in larvae is usually associated with transmission as a result of feeding on an infected vertebrate host.

The presented study shows that statistically significant dependency concerning the infected ticks was the season – the majority of the infected ticks was acquired from people who had been bitten in autumn ($p < 0.0001$), and accounted for 69.23% of the infected ticks. The authors found no similar research that could be used for analysis in this regard. The phenomenon of picking forest mushrooms in autumn, which is a fairly common custom in Poland, appears to be directly related to the results of this study; however, this assumption requires further observation and verification.

CONCLUSIONS

1. Infestation of ticks infected with Lyme disease spirochete in this study proved to be variable and depend on the season, the area of tick attack, and the region in Poland.
2. The results obtained in this study in relation to the form of development of ticks and the region from which they were collected also need to be highlighted. Statistical analysis showed an existing relationship between these variables. These results require further in-depth studies to help explain and understand such relationships.
3. The results of the study clearly show that ticks infected with *Borrelia burgdorferi* inhabit all regions of Poland. The results are consistent with National Institute of Hygiene data which indicates that Lyme disease cases are recorded in all regions of Poland

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