

OCCURRENCE OF *BORRELIA BURGDORFERI* SENSU LATO IN *IXODES RICINUS* AND *DERMACENTOR RETICULATUS* TICKS COLLECTED FROM ROE DEER AND DEER SHOT IN THE SOUTH-EAST OF POLAND

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Abstract

The investigations concerning the spirochaetes *Borrelia burgdorferi* sensu lato were conducted in 169 *Ixodes ricinus* female ticks (141 collected from roe deer and 28 from red deer) as well as in 11 *Dermacentor reticulatus* female ticks from deer shot in the South-East of Poland. Moreover, a single female *Dermacentor reticulatus* from a dog and 9 collected from plants were examined. DNA was isolated using the Genomic DNA Prep Plus kit. Its amplification was conducted by means of the diagnostic PCR-*Borrelia* kit. The presence of spirochaetes was observed in 31.3% of ticks collected from male roe deer, 21.4% - from female roe deer, and 32.1% - from red deer. The highest numbers of infected ticks were detected to date in the Lublin region. *Borrelia burgdorferi* was found in 5 (45.4%) *Dermacentor reticulatus* from deer and in single ticks from a dog and plants. These are the first cases in Poland, which indicate that *Dermacentor reticulatus* is involved in the transmission of borreliosis.

Key words: red deer, roe deer, *Borrelia burgdorferi*, *Dermacentor reticulatus*, *Ixodes ricinus*, Poland.

The present epidemiological situation of borreliosis in Poland is quite well known. Studies on the extensiveness of tick-borne infections with *Borrelia burgdorferi* were mainly conducted in the regions of Western Pomerania, Wielkopolska, and Lublin (3-5, 7-10, 12, 15, 17-22, 25, 26, 28). The extensiveness of borreliosis was also studied in humans, particularly in high-risk groups (1, 2, 6).

Nevertheless, numerous issues remain unexplained. One of them concerns the determination of various groups of hosts involved in the transmission of the pathogen (13, 16, 27). The majority of studies conducted so far dealt with ticks collected in the non-

parasite phase. The parasites that feed on the animal's blood may infect or become infected transmitting spirochaetes to the successive hosts. Borreliosis is transmitted in two planes: perpendicular (ticks themselves) and horizontal (between hosts and ticks). The infection in ticks is likely to persist for many generations due to transovarian and transtadial transmission of the spirochaetes. The female tick plays an essential role in the transmission of pathogens (14, 23, 25, 27); therefore, our study concerned only this stage of the parasite.

The studies conducted in Poland so far indicated that the only species of ticks involved in the borreliosis spread was *Ixodes ricinus* (4, 17, 25, 27).

The present study, the degree of *Borrelia burgdorferi* infection in *Ixodes ricinus* and *Dermacentor reticulatus* female ticks collected from *Capreolus capreolus* and *Cervus elaphus* shot in the Lublin region was investigated. Female ticks were the most common form of the parasites examined; other forms were rare.

Material and Methods

The study involved the females of *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from roe deer and red deer shot in the Lublin region and northern part of the Carpathian Foothills. Single parasites were randomly collected from host samples and kept in 70% ethyl alcohol until examinations.

In total, 169 *Ixodes ricinus* ticks were examined, including 99 collected from roe bucks in spring 2005, 42 from roe does (autumn 2005), 23 from deer bulls, and 5 from does (autumn 2005).

The *Dermacentor reticulatus* ticks (11) were obtained exclusively from deer bulls (autumn 2005).

Additionally, the female ticks from a dog and 9 females, collected from plants of the northern Lublin region (near Puławy and Lubartów) in 2006, were examined.

Detection of DNA of *Borrelia burgdorferi*.

The *Borrelia burgdorferi* spirochaetes in ticks were detected by the PCR method using a DNA amplification of the fla gene encoding the ciliary protein - flagellin. The following starters were used:

BFL1 5' GCT CAA TAT AAC CAA ATG CAC ATG 3'

BFL2 5' CAA GTC TAT TTT GGA AAG CAC CTA A 3'

The genome DNA was isolated from bacteria according to the Genomic DNA Prep Plus kit protocol (A&A Biotechnology, Poland). The isolated DNA was used in the PCR reaction (diagnostic kit PCR-Borrelia, DNA Gdańsk II).

The PCR solution contained: 20 µl of master mix, 2.5 µl of dNTP nucleotide mixture (2 mM), 0.5 µl of thermostable polymerase Delta 2, and 2.0 µl of DNA.

The DNA amplification reaction was conducted in the Mastercycler Personal (Eppendorf, Germany) – 40 cycles. The first initial denaturation was carried out at 93°C for 2 min. Each cycle involved: 30-s DNA denaturation at 93°C, 60-s addition of starters at 52°C, 60-s elongation of DNA chain at 72°C, and 60-s final elongation at 72°C.

The reaction products were separated in 2% agarose gel with ethidium bromide (Sigma Aldrich, Germany) during 1 h at 100 V. The marker of DNA size, DNA M1 (DNA Gdańsk) was used as a standard.

The amplification products were analysed in the UVP transilluminator (Eppendorf, Germany) and archived using the UVP software (Eppendorf, Germany). The expected size of the amplified gene fragment was 442 base pairs.

Results

***Ixodes ricinus* ticks.** In total, 169 female ticks from roe deer and red deer were examined. *Borrelia burgdorferi* was found in 49 (29%) of them (Table 1, Fig. 1). The infection extensiveness in roe deer parasites was 28.5% and in red deer – 32.1%.

Table 1

Extensiveness of *Borrelia burgdorferi* incidence in *Ixodes ricinus* females from roe deer and deer

Host	Number of examined ticks	Number of infected ticks (%)
Roe buck	99	31 (31.3)
Roe doe	42	9 (21.4)
Deer bull	23	8 (34.8)
Doe	5	1 (20)
Total	169	49 (29)

Lyme borreliosis was detected in 31 (31.3%) out of 99 females from roe bucks. The animals on which the infected parasites fed were shot in the region of Lublin, Chełm, Tarnogród, Kobylnica, Sieniawa, Horyniec, Strzelce, and Zalesie. In the regions, where ticks were collected, from at least 5 hosts the extensiveness of infection ranged from 22% (Lublin; in 2 of 9 examined), through 27% (Chełm, 9 of 33), 38% (Zalesie; 5 of 13) to 50% (Sieniawa; 5 of 10).

Negative results were obtained in ticks from roe bucks shot in the vicinity of Janów Lubelski, Tarnobrzeg, Narol, and Ustianowa near Ustrzyki Dolne.

The positive results were found in 9 (21.4%) of 42 ticks collected from roe deer shot in the vicinity of Krasnogliny near Dęblin (in 3 of 7 examined – 43%), Suchodoły near Kraśnik (3 of 8 – 37%), Leżajsk (1 of 9 – 11%), Chełm (1 of 3), and Krasnystaw (1). There were no *Borrelia* spirochaetes found in ticks collected from roe deer shot near Lublin, Zalesie, and Wisznice.

Amongst ticks obtained from 23 deer bulls, *Borrelia burgdorferi* was detected in 8 (34.8%). Only one tick of the 5 collected from does was infected. The presence of borreliosis was confirmed in the region of Zalesie, Opole Lubelskie, Chełm, Strzelce, Leżajsk, Adamówka, and Tarnogród.

***Dermacentor reticulatus* ticks.** DNA of *B. burgdorferi* was detected in 5 (45.4%) of 11 *Dermacentor reticulatus* samples collected from deer. The animals with infected parasites were from the region of Puławy, Chełm, Strzelce, Janów Lubelski, and Leżajsk. The infection was also detected in the tick from a dog and another one from plants of the western part of Lubartów. However, in the 8 remaining ones from Lasy Gułowskie, Lasy Kozłowieckie, and the Vistula valley, the bacterium was not found (Table 2).

Table 2

Extensiveness of *Borrelia burgdorferi* incidence in *Dermacentor reticulatus* females from hosts and plants

Place of collection	Number of examined ticks	Number of infected ticks (%)
Deer bull	11	5 (45.4)
Dog	1	1
Plants	9	1 (11)
Total	21	7 (33.3)

In deer, infestation with both tick species was observed in 8 cases – in 3 *Dermacentor reticulatus* females, 4 *Ixodes ricinus* ones; in one case the presence of bacteria in the tick corpse was not detected.

The study showed a relatively uniform distribution of borreliosis foci in the whole area examined (Fig. 2).

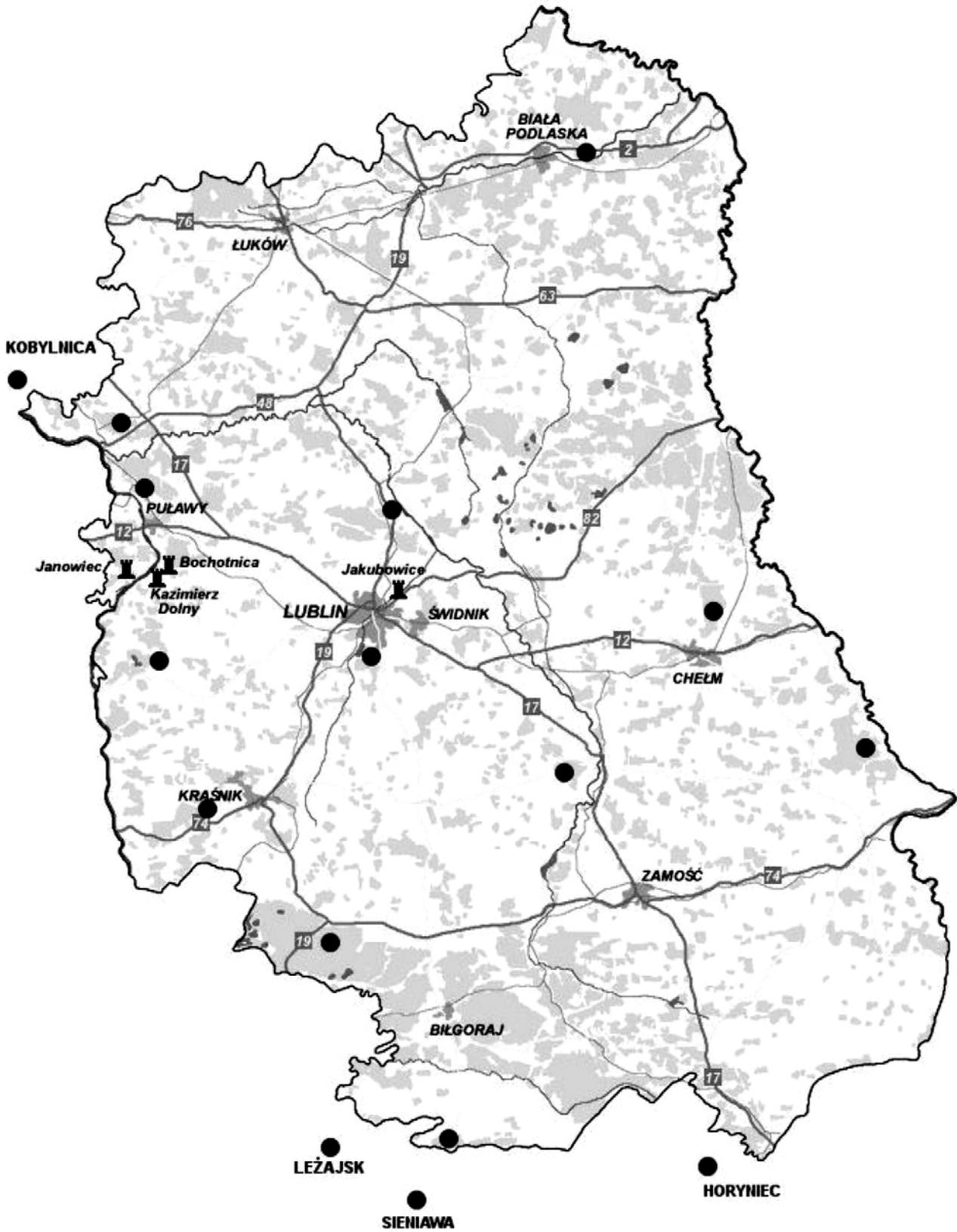


Fig. 2. The location of roe deer and red deer shooting where ticks infected with *Borrelia burgdorferi* spirochaetes were collected.

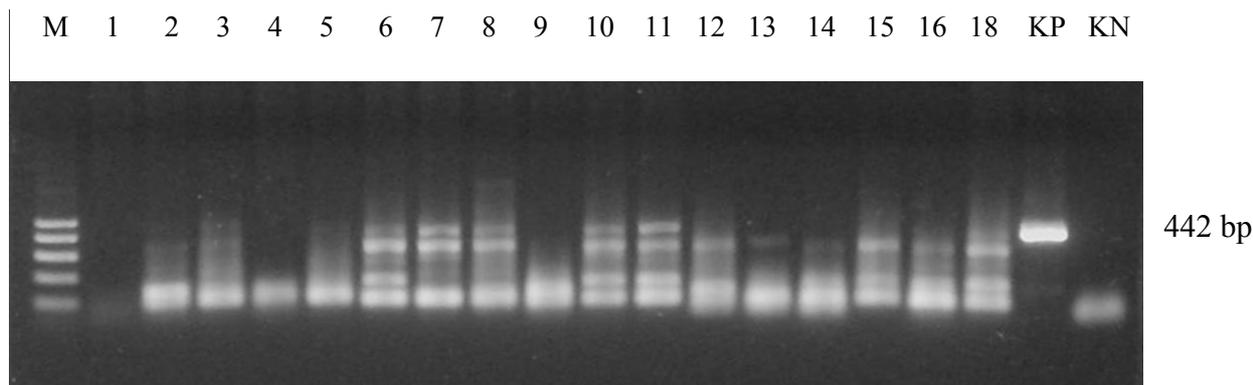


Fig. 1. PCR amplification product of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* on agarose gel after ethidium bromide staining. M – molecular size marker; lane 3, 6, 7, 8, 10, 11 and 18 with positive product of amplification, KP – positive control (442 bp), KN – negative control.

Discussion

The studies conducted in Poland showed that the extensiveness of spirochaete infection in *Ixodes ricinus* ticks is extremely varied. This might have been related to various factors, e.g. the method chosen, focal nature of the disease, poor representative character of samples, time of the tick's collection, and period of examinations (4, 8, 11, 14, 20, 21, 24, 29).

The results from the Lublin region confirm the regularities observed in the whole country. In several cases (5) negative results were obtained, in many others - highly variable. According to different authors, the average infection in various parts of the region was: 6.3% (3), 5.3% (4), and 11.3% (22). In the majority of cases, the highest values were detected in tick females. Our study demonstrated the highest (29%) mean percentage of *Borrelia* infections in tick females; particularly high values (34.8%), were found in parasites collected from deer bulls in autumn.

The data concerning *Borrelia burgdorferi* infections in ticks from hosts are not numerous. Tylewska-Wierzbanowska *et al.* (25) in their early studies demonstrated infections in 3.2% of *Ixodes ricinus* ticks collected from deer and wild boars. In their later studies, (25) the values found were even lower. Among 1 388 ticks collected from hosts in various parts of Poland in 1993-1995, only 12 (0.9%) were infected. Much higher values found in our study confirm an increase in borreliosis incidence observed recently in Poland, particularly east of the Vistula (1).

Small rodents are the essential source of infection (24), while birds are thought to be involved in the spread of the disease and development of its new foci (27). It has not been explained; however, whether large animal species are involved in the transmission of borreliosis. This is strictly connected with possible differences in the infection's severity of parasites collected from plants and various hosts. Tylewska-Wierzbanowska *et al.* (25) described the case from the Krosno region, in which borreliosis was diagnosed in 7

ticks from the dog, which indicates that its source was likely to be that dog. According to Wegner and Stańczak (27), large animals (mainly deer) are not capable of transmitting the spirochaetes to ticks, nevertheless their role in epidemiology is important as they are the main hosts of mature forms of ticks. Our findings seem to confirm this, at least partly, when two species of ticks were present on one deer, only one of them was infected.

The only known vector of *Borrelia burgdorferi* in Europe is *Ixodes ricinus* (27). According to Siński *et al.* (17), there are no known cases of *Borrelia burgdorferi* transmission by *Dermacentor reticulatus*. Studies of different authors conducted in Poland did not demonstrate the presence of bacteria in ticks collected from plants (4, 17) and hosts (5). Positive results were obtained only in the indirect immunofluorescence method, yet they were considered false (5).

The results of the present study reveal that *Dermacentor reticulatus* may be a vector of borreliosis. The spirochaetes were detected in ticks from both hosts (deer, dog) and plants. This is likely to be related to the expansion of *Dermacentor reticulatus* ticks in the Lublin region (our own studies), as well as increased Lyme borreliosis incidence in Poland (1).

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