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DETECTION OF *BORRELIA BURGDORFERI* SENSU LATO DNA IN *IXODES RICINUS* TICKS IN NORTH-WESTERN POLAND

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Abstract: In order to estimate the risk of contracting Lyme disease in the forest areas of north-western Poland, PCR-based studies were carried out on 6,817 *Ixodes ricinus* ticks for infection by the spirochaete *B. burgdorferi* sensu lato (s.l.). The studies were performed using the primers for the *fla* gene, conserved for all European genospecies of *B. burgdorferi* s.l. Based on the incidence of *B. burgdorferi* s.l. DNA in *I. ricinus* ticks at eight sampling sites during 1998–2001, it may be concluded that a risk of contracting Lyme disease is present in the forest areas of north-western Poland. The highest risk of infection (9.4% of infected ticks) is posed by human contact with female *I. ricinus*, and the risk is higher in late spring and early summer than in late summer and early autumn. The north-western part of Poland is an endemic region for *B. burgdorferi* s.l.

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Key words: DNA, *Borrelia burgdorferi* sensu lato, *Ixodes ricinus*, infection, north-western Poland.

INTRODUCTION

Borrelia burgdorferi is an etiological agent of Lyme disease (borreliosis), a multisystem zoonosis, one of the most important and world-wide distributed transmission diseases. The Ixodes tick is the vector of the bacterium, and in Europe, including Poland, the species *I. ricinus* tick is the vector. The disease, as well as the bacterium causing it, have been present in Europe at least since the 18th century [44], however, initially it was attributed to various other disease units. It was not until the end of the 1970s that the massive incidence of symptoms observed around the town of Old Lyme, USA, focused an attention on both the disease and the bacterium. Recently, several thousand cases of Lyme disease have been reported each year, both in Europe and in the USA [22].

The PCR method has been increasingly commonly used for detection of *B. burgdorferi* sensu lato (s.l.) DNA in tick vectors of Lyme disease spirochaetes [2, 11, 16, 19, 27, 29, 30]. Such studies are aimed at the determination of the degree of *Ixodes* tick population infection in particular areas and determination of endemic areas for *B. burgdorferi* s.l., and also represent an index of risk of contracting Lyme disease in those areas. The studies have been also carried on in Poland, covering a population of several hundred ticks from a single sampling site [13, 33, 36, 37, 38, 42, 43, 45].

This study is a continuation of the studies on the extensiveness of *I. ricinus* tick infection by *B. burgdorferi* s.l. in selected areas of the city of Szczecin and the West Pomeranian Province, using the PCR method, which have been carried on for several years at the Department of Genetics, University of Szczecin [37, 38, 39, 45]. The aim of this work was to determine the magnitude of risk of Lyme disease contracted by humans visiting forest areas of north-western Poland, through a four-year investigation of the incidence of infection in all the life stages of ticks that are *B. burgdorferi* vectors.

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MATERIALS AND METHODS

Material for the study, selection of seasons and sampling sites of *Ixodes ricinus* collection. The DNA of *B. burgdorferi* sensu lato was isolated from the intestinal content of 6,817 specimens of *I. ricinus* collected in north-western Poland. Each collected specimen was examined for life stage and sex of the adult form. *I. ricinus* ticks were collected using a flannel flag, with which the vegetation growing by forest roads was swept up to 1 m.

The ticks were collected during 1998-2001 in two seasons of their activity: spring-summer (May-June) and summer-autumn (August-October, later referred to as "spring" and "autumn") from eight forest sampling sites. Four sampling sites were set within the city of Szczecin: the Dąbie Forest Park, the Szczecin Landscape Park in Bukowa Forest, Arkoński Woods, and vicinity of lake Głębokie within Wkrzańska Forest. The remaining four sampling sites were located across the Province: Pobierowo, Goleniów Forest near the village of Rurka, the Ińsko Landscape Park, and Chojna (Fig. 1).

The assigned sampling sites are characterised by mixed stands of trees with common pine, oak, and common beech prevailing, also rich in undergrowth, thus being likely to create a habitat for *I. ricinus*. The examined sites belong to recreational areas in the north-western part of Poland, frequently visited by tourists and mushroom gatherers.

Borrelia burgdorferi sensu lato DNA was detected using polymerase chain reaction (PCR). The DNA of the bacteria from *I. ricinus* ticks was isolated with the method described by Stańczak *et al.* [42] and stored at -70ºC.

PCR conditions. A fragment of the *fla* gene, which encodes for the flagellum protein, flagellin, was the marker for *B. burgdorferi* s.l. DNA detection. A set of the following primers was applied: FLA1 (5' - AGA GCA ACT TAC AGA CGA AAT TAA T - 3') and FLA2 (5' - CAA GTC TAT TTT GGA AAG CAC CTA A - 3'), being complementary to the region of the gene *fla*, conserved for all five European species of *B. burgdorferi* s.l., derived through a comparison of nucleotide sequence of the *fla* gene of the Borrelia spirochaetes collected in the GenBank (EMBL - GenBank) [47]. The PCR product was of 482 bp in length.

The 20 µl PCR mixture contained 0.5 U of Taq DNA polymerase - recombinant (MBI Fermentas, Lithuania), 75 mM of Tris-HCl (pH 8.8 at 25ºC), 20 mM of (NH4)2SO4, 0.01% of Tween 20, 50 µM of each trideoxynucleotide, 1.5 mM of MgCl2, 400 pmol of each primer (FLA1 and FLA2), and 2 µl of DNA isolated from the ticks. The DNA of the Bo-148c/2 strain of *B. burgdorferi* s.s., (obtained by courtesy of Dr. Stańczak, identified at the Loyola University Medical Center, Maywood, Illinois, USA) [40], was used as the positive control. TE buffer (pH 8.0) was used as the negative control.

Figure 1. Collection sites of *I. ricinus*. DB - Dąbie Forest Park, SP - Szczecin Landscape Park, GŁ - Głębokie, AR - Arkonka, GO - Goleniów Forest, PB - Pobierowo, IN - Ińsko Landscape Park, CH - Chojna, doted line - border of former province of Szczecin.

The PCR was performed in thermocyclers: T-gradient (Biometria, Germany) and Peltier Thermal Cycler 200 (MJ Research Inc., USA). The temperature profile was the same as previously described [47].

The PCR products were separated on 2% agarose gel (ICN, USA) with addition of ethidium bromide (Sigma-Aldrich, Germany) at 90 V for 45 minutes. MW501 mass marker (Polgen, Poland) was applied for evaluation of the mass of the obtained product. The results of the PCR were viewed under UV light and archived in computer storage using BioCapt software (Vilber Lourmat, France).

Statistical analysis of results. The chi-square test included in the Statistica 6.0 (StatSoft, Inc., 2001, data analysis software system), was applied for the statistical analysis of *B. burgdorferi* s.l. spirochaete infection incidence by the stages of *I. ricinus* at individual sampling sites during the four years, in the spring and autumn seasons. The analysis covered the incidence of infection calculated as a percentage at a given sampling site in subsequent years, the seasons included. The analysis also included the infection incidence calculated as a percentage at a given sampling site in spring and autumn in subsequent years, and the mean incidence of infection calculated as a percentage at individual sampling sites for the total collection, divided by seasons.

RESULTS

***Borrelia burgdorferi* sensu lato DNA in *Ixodes ricinus* ticks.** The DNA of the spirochaete *B. burgdorferi*

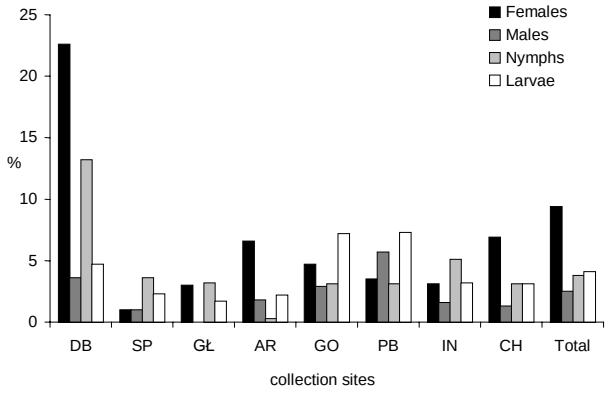

Figure 2. Occurence of *B. burgdorferi* sensu lato DNA (%) in *I. ricinus* ticks by stages and sexes at individual sampling sites during 1998–2001; DB - Dąbie Forest Park, SP - Szczecin Landscape Park, GŁ - Głębokie, AR - Arkonka, GO - Goleniów Forest, PB - Pobierowo, IN - Ińsko Landscape Park, CH - Chojna.

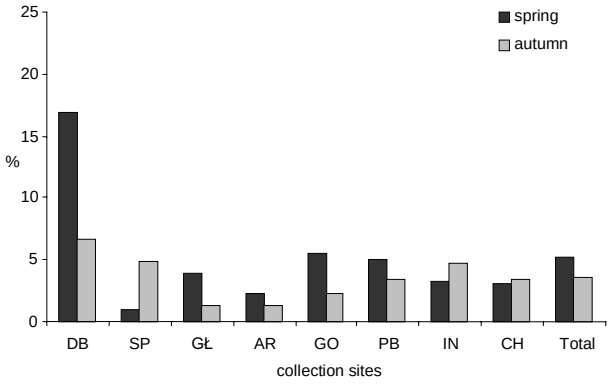

Figure 3. Occurence of *B. burgdorferi* sensu lato DNA (%) in *I. ricinus* ticks in spring and autumn at individual sampling sites during 1998-2001; DB - Dąbie Forest Park, SP - Szczecin Landscape Park, GŁ - Głębokie, AR - Arkonka, GO - Goleniów Forest, PB - Pobierowo, IN - Ińsko Landscape Park, CH - Chojna.

s.l. was detected in 299 isolates, which was 4.4% of the studied population (Tab. 1 and 2). The DNA of *B. burgdorferi* was found in 9.4% of females, in 3.8% of nymphs, 4.1% of larvae, and in 2.5% of males (Fig. 2). In spring, the infection was found in 5.2% of the studied population of *I. ricinus*, while in late summer and early autumn, 3.5% were infected (Fig. 3). The mean percentage of infection for 4 years at the site Dąbie Forest Park sampling was 11.9%, and ranged between 4.0–4.2% at four other sampling sites (Ińsko Landscape Park, Goleniów Forest, Pobierowo). In the remaining four sampling sites, the infection incidence ranged from 1.7% (Arkoński Woods) to 3.3% (Chojna, Tab. 1 and 2). The results of *B. burgdorferi* s.l. DNA incidence at individual sampling sites, in relation to stages and collection seasons in subsequent years 1998–2001, are presented in Tables 1 and 2, as well as in Figures 2–4.

A comparison of *I. ricinus* incidence of infection with the spirochaete *B. burgdorferi* s.l. in individual years at all the studied sampling sites, expressed in percent, did not display statistically significant differences ($p > 0.052$). Statistically significant differences were found at the Dąbie Forest Park sampling site in spring and autumn seasons, comparing the rate of infections in 2001 with the other three years ($p < 0.03$). Statistically significant differences were also found in spring at the Głębokie and Pobierowo sampling sites, compared to infections in 1999 and 2001 ($p < 0.04$) and in autumn at the Szczecin Landscape Park sampling site, in 1999 and 2001 ($p < 0.01$). A comparison of infection incidence in both seasons of *I. ricinus* collection at all the studied sampling sites demonstrated statistically significant differences at four sampling sites: Dąbie Forest Park, Szczecin Landscape Park, Ińsko Landscape Park, and Pobierowo. Between the spring and autumn seasons, the statistically significant differences were found: in all four years at the Dąbie Forest Park ($p < 0.01$). At the remaining three sampling sites, the differences were found: at Szczecin Forest Park in 1999 ($p = 0.0014$), at Ińsko Landscape Park in 2000 ($p = 0.037$), and at Pobierowo in 2001 ($p = 0.009$).

A comparison of *I. ricinus* infection rate at particular sampling sites revealed statistically significant differences at the Dąbie Forest Park as compared with the remaining sampling sites.

DISCUSSION

The studies covered the population of the *I. ricinus* tick which, as a vector of *B. burgdorferi* s.l., represents a source of information on the risk of infection for humans and animals. *I. ricinus* belong to precocial ticks, which are of low-motility, settle usually on vegetation, and passively wait for an accidental occurrence of a moving host [35]. Due to this fact, the collection of ticks was carried out in places of the highest probability of encountering *I. ricinus*, i.e. at the edge of forest paths. Polish populations of *I. ricinus* display two seasons of peak activity of host-seeking individuals, i.e. in spring (spring peak) and in late summer/early autumn (autumn peak) [35], therefore the collection at each sampling site was performed twice a year, with the seasons of activity taken into account, i.e. at a time of the highest risk for a human to be bitten by a tick.

B. burgdorferi s.l. DNA was most frequently detected in females, less frequently in nymphs and larvae, and least frequently in males (Fig. 2). Each stadium of the tick may be a carrier of the spirochaete *B. burgdorferi* s.l., contract it through a transovarial (larvae) or transstadial (nymphs and adult form) passage, whereas feeding intensity increases the chance of spirochaete contracting and their further transmission to subsequent stadiums. Therefore, *B. burgdorferi* s.l. was most frequently detected in adult forms, especially in females. In host-seeking ticks, the spirochaete *B. burgdorferi* s.l. infest the intestine. In adult forms, a part of the spirochaetes wander to the gonads, while a small part remain in the intestine where they proliferate before feeding [10]. The males of *I. ricinus* seldom feed, and their activity is usually in search of

females. A low degree of infection in males may result from the fact that much less *B. burgdorferi* s.l. spirochaetes

Table 1. Prevelance of DNA of *B. burgdorferi* s.l. (PCR+, N and %) in different stages of *I. ricinus* ticks in selected forested areas of north-western Poland in 1998-2001 (N - number of ticks collected, n - number of PCR-positive samples).

Collection site	Year	Females			Males			Nymphs			Larvae			Total		
				PCR+			PCR+			PCR+			PCR+			PCR+
		N	n	%	N	n	%	N	n	%	N	n	%	N	n	%
Dąbie Forest Park	1998	76	19	25	54	1	1.9	64	6	9.4	17	1	5.9	211	27	12.8
	1999	53	18	34	41	2	4.9	71	8	11.3	44	4	9.1	209	32	15.3
	2000	64	19	29.7	55	2	3.6	38	2	5.3	73	2	2.7	230	25	10.9
	2001	64	2	3.1	70	3	4.3	53	14	26.4	36	1	2.8	223	20	9
	subtotal	257	58	22.6	220	8	3.6	226	30	13.2	170	8	4.7	873	104	11.9
Szczecin Landscape Park	1998	43	1	2.3	42	1	2.4	111	3	2.7	11	1	9.1	207	6	2.9
	1999	9	0	0	15	0	0	132	9	6.8	33	0	0	189	9	4.8
	2000	25	0	0	19	0	0	143	5	3.5	13	0	0	200	5	2.5
	2001	28	0	0	24	0	0	136	2	1.5	30	1	3.3	218	3	1.4
	subtotal	105	1	1	100	1	1	522	19	3.6	87	2	2.3	814	23	2.8
Głębokie	1998	31	0	0	28	0	0	114	4	3.5	20	0	0	193	4	2.1
	1999	23	2	8.7	22	0	0	144	8	5.6	20	1	5	209	11	5.3
	2000	16	1	6.3	6	0	0	165	5	3	6	0	0	193	6	3.1
	2001	30	0	0	30	0	0	142	1	0.7	13	0	0	215	1	0.5
	subtotal	100	3	3	86	0	0	565	18	3.2	59	1	1.7	810	22	2.7
Arkoński Woods	1998	27	1	3.7	12	0	0	50	0	0	99	3	3	188	4	2.1
	1999	18	1	5.6	8	0	0	104	0	0	85	2	2.4	215	3	1.4
	2000	21	0	0	12	1	8.3	129	1	0.8	19	0	0	181	2	1.1
	2001	35	4	11.4	23	0	0	94	0	0	71	1	1.4	223	5	2.2
	subtotal	101	6	6.6	55	1	1.8	377	1	0.3	274	6	2.2	807	14	1.7
Goleniów Forest	1998	13	0	0	13	0	0	129	6	4.7	44	0	0	199	6	3
	1999	27	1	3.7	19	2	10.5	152	5	3.3	34	0	0	232	8	3.4
	2000	22	0	0	8	0	0	139	5	3.6	40	2	5	209	7	3.3
	2001	24	3	12.5	28	0	0	98	0	0	48	10	20.8	198	13	6.6
	subtotal	86	4	4.7	68	2	2.9	518	16	3.1	166	12	7.2	838	34	4.1
Pobierowo	1998	13	0	0	11	0	0	136	2	1.5	68	8	11.8	228	10	4.4
	1999	26	0	0	33	0	0	135	6	4.4	58	1	1.7	252	7	2.8
	2000	27	1	3.7	39	2	5.1	139	5	3.6	6	0	0	211	8	3.8
	2001	25	2	8	22	4	18.2	146	4	2.7	46	4	8.7	239	14	5.9
	subtotal	91	3	3.5	105	6	5.7	556	17	3.1	178	13	7.3	930	39	4.2
Ińsko Landscape Park	1998	12	0	0	20	0	0	106	5	4.7	68	3	4.4	206	8	3.9
	1999	7	0	0	10	0	0	144	9	6.3	55	2	3.6	216	11	5.1
	2000	16	1	6.3	37	1	2.7	112	7	6.3	30	0	0	195	9	4.6
	2001	61	2	3.3	60	1	1.7	111	3	2.7	4	0	0	236	6	2.5
	subtotal	96	3	3.1	127	2	1.6	473	24	5.1	157	5	3.2	853	34	4
Chojna	1998	20	0	0	12	0	0	125	2	1.6	47	1	2.1	204	3	1.5
	1999	30	4	13.3	32	0	0	124	4	3.2	34	3	8.8	220	11	5
	2000	13	1	7.7	21	1	4.8	90	5	5.6	117	3	2.6	241	10	4.1
	2001	9	0	0	12	0	0	114	3	2.6	92	2	2.2	227	5	2.2
	subtotal	72	5	6.9	77	1	1.3	453	14	3.1	290	9	3.1	892	29	3.3
Total	1998	235	21	8.9	192	2	1	835	28	3.4	374	17	4.5	1636	68	4.2
	1999	193	26	13.5	180	4	2.2	1006	49	4.9	363	13	3.6	1742	92	5.3
	2000	204	25	12.3	197	7	3.6	955	35	3.7	304	7	2.3	1660	72	4.3
	2001	276	13	4.7	269	8	3	894	27	3	340	19	5.6	1779	67	3.9
	total	908	85	9.4	838	21	2.5	3690	139	3.8	1381	56	4.1	6817	299	4.4

remain in their intestines compared to females, and therefore are more difficult to be detected.

In spring, the infection incidence of *I. ricinus* population was higher than in late summer/early autumn (Fig. 3), and the differences were statistically significant. Also, in individual years the incidence of ticks infection was higher in spring than in autumn (Tab. 2). The mean percentage of infection rate for four years was the highest at the Dąbie Forest Park (Tab. 1 and 2, Fig. 4), and the infection of ticks by the *B. burgdorferi* s.l. spirochaete was the highest at this sampling site in individual years, and the differences were statistically significant in relation to the other seven sampling sites.

The degree of ticks infection was generally higher in spring than in autumn in subsequent years; however, at particular sampling sites the incidences in spring were not always higher than in autumn, even at the same sampling site (Tab. 2). Only at the Głębokie sampling site, in all the years, in spring, the *I. ricinus* infection rate by *B. burgdorferi* s.l. was higher than in autumn. At the remaining seven sampling sites, the infection incidence in ticks was varied for both seasons of their activity in individual years (Tab. 2). The regularly occurring higher level of *I. ricinus* infection by *B. burgdorferi* s.l. in spring at one sampling site, and a variable infection degree at the remaining sampling sites, may result either from a variety of animal reservoir of Lyme disease spirochaetes at individual sampling sites, or from climatic conditions and habitats, which may significantly influence the activity of tick and, hence, the degree of their infestation in particular seasons and years.

A comparison of the degree of *I. ricinus* ticks infection by *B. burgdorferi* s.l. in particular years at all the studied sampling sites did not demonstrate any statistically significant differences. This means that the rate of *I. ricinus* ticks infection by *B. burgdorferi* s.l. at individual sampling sites is comparable over the subsequent years. A comparison of *I. ricinus* infection rate in both seasons of collection at all the studied sampling sites demonstrated statistically significant differences at four sampling sites: Dąbie Forest Park, Szczecin Landscape Park, Ińsko Landscape Park, and Pobierowo. Between the spring and autumn seasons, the statistically significant differences occurred in all four years at the sampling site Dąbie Forest Park, which means an occurrence of a relationship between the rate of *I. ricinus* ticks infection by *B. burgdorferi* s.l. and the season of activity at this sampling site. As no significant differences were found, at the remaining sampling sites, no seasonal dependence can be demonstrated in the rate of *I. ricinus* ticks infection by *B. burgdorferi* s.l. A comparison of the degree of *I. ricinus* ticks infection at particular sampling sites revealed statistically significant differences at the Dąbie Forest Park where the highest mean infection rate in four years was detected, as compared with the remaining sampling sites. This enables a conclusion that a relationship exists between the rate of *I. ricinus* ticks infection by *B. burgdorferi* s.l. and the sampling site of collection.

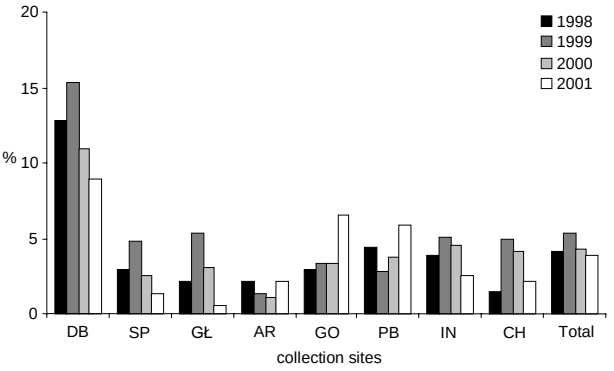

Figure 4. Occurence of *B. burgdorferi* sensu lato DNA (%) in *I. ricinus* ticks in subsequent years 1998-2001; DB - Dąbie Forest Park, SP - Szczecin Landscape Park, GŁ - Głębokie, AR - Arkonka, GO - Goleniów Forest, PB - Pobierowo, IN - Ińsko Landscape Park, CH - Chojna.

According to data published in Morbidity and Mortality Weekly Report in 1990, a region is considered endemic for Lyme disease where at least two cases of the disease in humans were detected and confirmed, and the occurrence of *B. burgdorferi* spirochaetes was detected in ticks (Morbidity and Mortality Weekly Report, January 28, 1991, after Pancewicz *et al.* [23]). Since 1987, when 14 cases of Lyme disease among inhabitants of north-western Poland was described for the first time, the region has been recognized as an endemic region [12]. The results of *I. ricinus* in the presented study, as well as those published previously [36, 37, 47], confirm that the north-western part of Poland is an endemic region for *B. burgdorferi* s.l.

The results achieved in this study also provide evidence for a considerable variability in the infection rate of *I. ricinus* across north-western Poland, as well as confirm previous studies on the incidence of the spirochaete *B. burgdorferi* s.l. in *I. ricinus* ticks, based on the gene *fla*, and carried on at the Department of Genetics, University of Szczecin, since 1996 [36, 37]. This particularly refers to the Dąbie Forest Park, where a high incidence of *I. ricinus* infection were reported in the spring seasons of 1996 and 1997 (9.8% and 19.2% respectively). The results of the investigations in the presented study also provide confirmation of *B. burgdorferi* s.l. presence, detected in Polish populations of ticks using the PCR method.

The DNA of *B. burgdorferi* s.l. was the least frequently detect in *I. ricinus* in the areas of the former voivodships: Krosno, Koszalin, and Suwałki (0.77%) [45]. A higher infection rate of *I. ricinus* was observed in the area of Bydgoszcz, Słupsk, Lublin, Gorzów, Kraków, and Poznań (5.3–22.6%), and highest in the vicinity of Katowice and Konin (37.5–46.4%) [7, 13, 20, 42].

Similar results, based on the PCR method, were obtained in other European countries. The lowest incidence of the spirochaete *B. burgdorferi* s.l. infection of *I. ricinus* ticks was recorded in the Czech Republic during 1995–1998 (2.8–9.2%). A degree of imago infection was higher than in nymphs [3]. A higher rate of infected *I. ricinus*

Table 2. Prevalence of DNA of *B. burgdorferi* sensu lato (PCR+, n and %) in *I. ricinus* ticks at sampling sites in spring and autumn during 1998–2001 (N - number of ticks collected, n - number of PCR-positive samples).

Collection site	season	1998			1999			2000			2001			Total		
			PCR+			PCR+			PCR+			PCR+			PCR+	
		N	n	%	N	n	%	N	n	%	N	n	%	N	n	%
Dąbie Forest Park	spring	100	25	25	102	26	25.5	130	21	16.2	118	4	3.4	450	76	16.9
	autumn	111	2	1.8	107	6	5.6	100	4	4	105	16	15.2	423	28	6.6
	total	211	27	12.8	209	32	15.3	230	25	10.9	223	20	9	873	104	11.9
Szczecin Landscape Park	spring	104	1	1	98	0	0	109	1	0.9	113	2	1.8	424	4	0.9
	autumn	103	5	4.9	91	9	9.9	91	4	4.4	105	1	1	390	19	4.9
	total	207	6	2.9	189	9	4.8	200	5	2.5	218	3	1.4	814	23	2.8
Głębokie	spring	101	3	3	115	9	7.8	105	4	3.8	110	1	0.9	431	17	3.9
	autumn	92	1	1.1	94	2	2.1	88	2	2.3	105	-	-	379	5	1.3
	total	193	4	2.1	209	11	5.3	193	6	3.1	215	1	0.5	810	22	2.7
Arkoński Woods	spring	105	3	2.9	108	1	0.9	89	1	1.1	114	4	3.5	416	9	2.2
	autumn	83	1	1.2	107	2	1.9	92	1	1.1	109	1	0.9	391	5	1.3
	total	188	4	2.1	215	3	1.4	181	2	1.1	223	5	2.2	807	14	1.7
Goleniów Forest	spring	105	3	2.9	114	6	5.3	98	5	5.1	129	11	8.5	446	25	5.6
	autumn	94	3	3.2	118	2	1.7	111	2	1.8	69	2	2.9	392	9	2.3
	total	199	6	3	232	8	3.4	209	7	3.3	198	13	6.6	838	34	4.1
Pobierowo	spring	118	4	3.4	112	3	2.7	99	3	3	126	13	10.3	455	23	5.1
	autumn	110	6	5.5	140	4	2.9	112	5	4.5	113	1	0.9	475	16	3.4
	total	228	10	4.4	252	7	2.8	211	8	3.8	239	14	5.9	930	39	4.2
Ińsko Landscape Park	spring	99	2	2	112	8	7.1	109	2	1.8	132	3	2.3	452	15	3.3
	autumn	107	6	5.6	104	3	2.9	86	7	8.1	104	3	2.9	401	19	4.7
	total	206	8	3.9	216	11	5.1	195	9	4.6	236	6	2.5	853	34	4
Chojna	spring	105	1	1	108	5	4.6	122	6	4.9	110	2	1.8	445	14	3.1
	autumn	99	2	2	112	6	5.4	119	4	3.4	117	3	2.6	447	15	3.4
	total	204	3	1.5	220	11	5	241	10	4.1	227	5	2.2	892	29	3.3
Total	spring	837	42	5	869	58	6.7	861	43	5	952	40	4.2	3519	183	5.2
	autumn	799	26	3.3	873	34	3.9	799	29	3.6	827	27	3.3	3298	116	3.5
	total	1636	68	4.2	1742	92	5.3	1660	72	4.3	1779	67	3.9	6817	299	4.4

specimens was reported in Ireland, Sweden, and Belgium (14.9–23.3%), where the DNA of *B. burgdorferi* s.l. was more often detected in adult forms than in nymphs [5, 16, 17, 19]. In Holland, the level of infection ranged between 13.2–15.6%; however the DNA of *B. burgdorferi* s.l. was more often detected in nymphs than in adult forms [28, 29, 30, 31]. In Germany, the rate of infection within the entire studied population was relatively high (15.7–35.%), and the infection rate in nymphs was lower than in adult forms [4, 8, 27, 46].

A high rate of *I. ricinus* infection by *B. burgdorferi* (32%) was also recorded in Finland [15]. In the United Kingdom, the infection rate of tick ranged from 4.1–34.2%, and considerable differences in the infection rate were also detected between nymphs and adult forms [18]. The highest incidences of infected *I. ricinus* were found in Italy (40%) and in Croatia (45.2%) and it was slightly lower in nymphs than in adult forms [6, 29]. In France, where the tick infection rate was 12%, the studies covered nymphs only [25, 26].

Various incidence of infection in European populations of *I. ricinus* by *B. burgdorferi* s.l. spirochaete, obtained with the PCR method, may have several reasons. The fundamental one may lie in the still unknown animal host-reservoir of the Lyme disease spirochaete. At present, nine species of small mammals and seven medium-sized mammals, as well as 16 species of birds, including Ploceidae, marine birds, and pheasants, are considered able to transmit spirochaetes to ticks and, this way, to participate in the natural circulation of *B. burgdorferi* s.l. in Europe [9]. Also, the factors controlling the activity of the bacterium's vector, including the number of peaks in the activity of ticks, which depends on the latitude of their habitat or weather changes that disturb natural seasonal

and diel rhythms of *I. ricinus*, may influence the spreading of the spirochaetes and their survival rate in ticks [1, 14, 24, 32, 35].

The obtained results of infection incidence may also be influenced by the size of the studied population of ticks, as the quoted data were obtained for the population of several tens to several hundreds of specimens. Another reason may be that the methods of *B. burgdorferi* DNA detection in ticks have not been unified. Application of various markers, as well as various primers for the same markers, influences the sensitivity of the reaction and result differentiation [2, 21, 34, 41]. Bearing all the above factors in mind, the results of *I. ricinus* infection obtained around Europe cannot be considered as ultimate, but should be treated as approximate only, as an estimation of the risk of contracting Lyme disease.

The results obtained in this study demonstrate that there is a risk of contracting Lyme disease in the area of north-western Poland, the highest being due to contact with females, lower as a result of a contact with nymphs and larvae, and the lowest with males. The risk is higher in spring than in autumn. The north-western part of Poland is an endemic region for *B. burgdorferi* s.l.

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