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THE OCCURRENCE OF *BORRELIA BURGDORFERI* SENSU LATO IN THE POPULATIONS OF *IXODES RICINUS* IN FOREST AREAS OF SZCZECIN DURING 2000–2001

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Abstract: The aim of the present study was to determine the seasonal abundance of ticks, *Ixodes ricinus* and their infection frequency with spirochetes, *Borrelia burgdorferi* in wooded areas of the vicinity of the city of Szczecin during 2000–2001. The ticks were collected from April to October. Presence of *Borrelia burgdorferi* sensu lato was detected with the method of indirect immunofluorescence (IFA). The results showed differences in seasonal activity of ticks within the years of the study. In the year 2000 a single peak of their increased activity was noted, while in the year 2001 no significant changes in the population of *Ixodes ricinus* were recorded. *Borrelia burgdorferi* s. l. was detected in 2000 and 2001 in 11.6% and 9.6% of the specimens captured, respectively, which constituted a statistically significant difference. The highest level of infection of *Ixodes ricinus* in 2000 was observed in April (13.2%), while in 2001 - in July (12.7%). The differences in the prevalence value between respective months of both years were not statistically significant. The results of the present study indicate a high risk factor of acquiring Lyme borreliosis by humans and animals in wooded areas of the Szczecin vicinity.

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INTRODUCTION

First cases of Lyme borreliosis in Poland were recorded in the 1980s in the area of Western Pomerania [9]. A steady increase of the number of cases has been observed ever since. Relevant epidemiological studies indicated that forest rangers were the professional group representing the highest risk of acquiring the disease [3, 12, 18, 19, 27].

Many research centres in Poland conduct monitoring studies on the persistence of the aetiological agent of the Lyme disease in the population of the vector - *Ixodes ricinus* [4, 6, 7, 8, 9, 10, 11, 13, 16, 20, 21, 22, 23, 25, 26, 28, 29, 30, 31, 32, 33, 34] and also in the populations of reservoir hosts: mammals and birds [2, 17] and reptiles [1].

The aim of the present paper was the assessment of seasonal changes in relative density of ticks and in their infection levels with spirochetes, *Borrelia burgdorferi* in the north-western Poland.

MATERIAL AND METHODS

Ticks were collected from 10 sites in the municipal forests of the city of Szczecin. Each site covered an area of 100 m^2. Ticks were collected from underbrush and forest bedding using a 1 m^2 piece of a flannel cloth. The procedure was repeated once a month from April to October in 2000 and 2001. A total of 2380 ticks were covered in this study. This number included 2195 nymphs

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and 185 adults (89 females and 96 males). The larvae were not used for further study because of their low survival rate.

The spirochetes were detected using the method of indirect immunofluorescence. Each tick was rinsed in 70% ethyl alcohol, dried, and squashed with a glass rod. It was then placed in a buffered physiological solution (PBS). In next step, 10 µl of the suspension was transferred to an immunofluorescence slide and after drying it was fixed in acetone. Such prepared samples were bind to anti-*Borrelia burgdorferi* rabbit antibodies and with anti-rabbit goat IgG marked with fluorescein isothiocyanate (FITC). The results of IFA reaction in the form of glowing spirochete–rabbit antibody–goat marked antibody complexes were observed under a fluorescence microscope under magnification of × 400.

Chi-square test of independence was used for statistical processing of the results. Differences with $p \leq 0.05$ were considered as statistically significant. To demonstrate possible correlation between the quantity and the infection level of ticks Spearman's rank correlation coefficients were calculated. Computer software "Statistica 99" was used for data processing.

RESULTS

In the year 2000 a total of 1387 ticks were collected. In this number nymphs constituted 91.1%, whereas the adults were represented by 8.9% of the specimens (4.5% females, 4.4% males) (Tab. 1). In the same year a single peak of *I. ricinus* activity was observed in June (Fig. 1). The quantitative differences of *Ixodes ricinus* between June and the remaining months were statistically significant. Tick numbers collected in individual months of 2000 varied significantly, except for September, October, April and May.

The year 2001, compared to the previous year, yielded substantially fewer ticks from designated sites - a total of 993 specimens (Tab. 1), which constituted a statistically significant difference. The nymphs constituted 93.7% of the overall number of the ticks collected, whereas the adults were represented by 6.2% of the specimens (2.7% females, 3.5% males) (Tab. 1). The numbers of *Ixodes ricinus* collected in individual months of 2001 did not show statistically significant differences ($p > 0.05$).

The highest difference recorded was between August and October and the significance level for this difference reached the value of $p = 0.07$.

Statistical differences were demonstrated between May and June while comparing the numbers of the specimens collected in respective months of 2000 and 2001 (Fig. 1).

The presently conducted reactions of indirect immunofluorescence showed 11.6% infection level of the tick, *Ixodes ricinus* population with spirochetes *Borrelia burgdorferi* in 2000 and 9.6% in the year 2001 (Tab. 1). The two-year study demonstrated also a higher percentage of infected specimens in the adults than in nymphs. *B. burgdorferi* sensu lato spirochetes were detected in 19.5% of the adult specimens in 2000 and in 24.2% of them in the following year. The infection level of males and females was similar (Tab. 1). The percentage of infected nymphs was 10.8% and 8.6% respectively (Tab. 1).

Table 1. Infection levels of ticks, *Ixodes ricinus* with spirochetes *Borrelia burgdorferi* sensu lato in 2000 and 2001

Developmental stage	Number of infected ticks/ Number of examined ticks		Prevalence of *B. burgdorferi* s. l.	
	2000	2001	2000	2001
Nymphs	137/1264	80/931	10.8	8.6
Females	12/62	7/27	19.3	25.9
Males	12/61	8/35	19.7	22.8
Total	161/1387	95/993	11.6	9.6

Table 2. Correlation between infection level of ticks, *Ixodes ricinus* with spirochetes *Borrelia burgdorferi* sensu lato and the quantitative size of the population.

Year	R_S	p
2000	0.85	0.02*
2001	0.53	0.2

R_s - Spearman's rang correlation coefficient; P - significance indicator; * - statistically significant value ($p \leq 0.05$)

The highest monthly prevalence in 2000 was observed in April, while the lowest - in May (Fig. 2). In 2001 the highest prevalence was recorded in July (Fig. 2), while the lowest - in August (Fig. 2). Those differences, in both years, were not statistically significant ($p > 0.05$). Also no statistical differences were noted between respective months of both years.

Statistically significant correlation coefficient between the number of ticks collected and their level of infection with *Borrelia burgdorferi* s.l. was stated only in 2000 (Tab. 2).

DISCUSSION

The results of the present study, as well as the results published by the other authors, show different quantitative dynamics of the *Ixodes ricinus* populations in different areas and different years. In this study, in 2000, there was a distinct peak of seasonal activity in June, while in 2001 the numbers of tick populations did not differ significantly between April and October.

The majority of authors, who conducted similar studies in other regions of Poland and also in other countries, observed most often two periods of increased tick activity: spring, lasting from April to May, and autumn - in September (for example Nowosad *et al.* [20] in the city limits of Poznań and Wegner *et al.* [34] in the vicinity of Olsztyn. Similar changes in the population quantities of *I. ricinus* throughout the year were observed by Talleklint and Jaenson [30] in Sweden, Pichon *et al.* [21] in wooded areas of France, and Ročkiene [24] in Lithuania. The present results differed from those published by the above-mentioned authors by showing only one peak of intensified

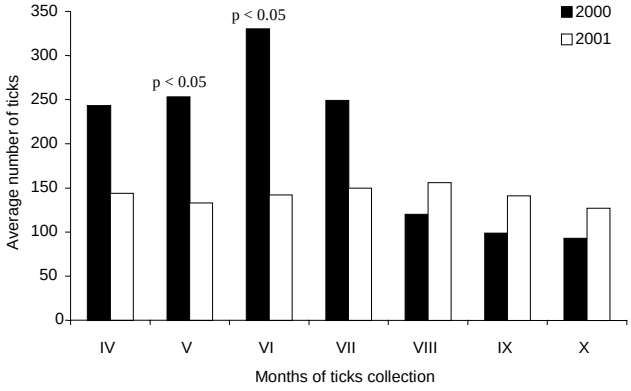

Figure 1. Comparision of the seasonal activity dynamics of *Ixodes ricinus* population during 2000–2001. Average number of ticks colleted from 10 sites of 100 m^2.

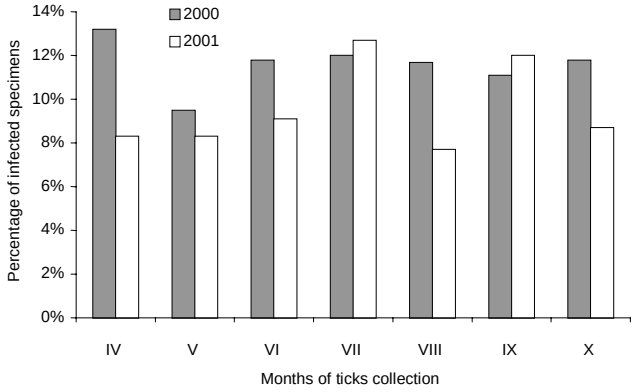

Figure 2. Comparision of the infection level dynamics of *Ixodes ricinus* population with *Borrelia burgdorferi* sensu lato during 2000–2001.

activity of *I. ricinus* in the month of June (Fig. 1) and no activity differences of *Ixodes ricinus* in 2001. Also, the number of ticks in September was among the lowest. A similar situation with no increase of tick population in autumn was observed in certain years in the vicinity of the Gdańsk-Gdynia-Sopot agglomeration, by Wegner *et al.* [31]. The above authors also recorded a summer increase of *I. ricinus* activity in June, July, or August, depending on the year and the area where ticks were collected.

Similar results, with the use of IFA method, were obtained Wegner *et al.* [34] in the area of the former Olsztyńskie Province in 1993 (tick prevalence of 11.5%) and Wegner *et al.* [32] on recreational areas of the former Białostockie Province in 1994 (8.8% of *I. ricinus* infected). Using the same technique Stańczak *et al.* [28] detected presence of *B. burgdorferi* in 8.1% of ticks in the areas of Białystok, Olsztyn and Elbląg in 1996.

Also in the studies based on PCR, results comparable with the present results were obtained. Stańczak *et al.* [28] recorded 13% infection of ticks collected in 1998 from the areas of Słupsk, Bydgoszcz, Lublin, and Kraków. Skotarczak and Wodecka [26] observed 12% infection of *I. ricinus* collected within the Zachodniopomorskie Province in 1996, while Skotarczak [25] noted the presence of the spirochetes in 8.6 % of the specimens collected in the same areas in 1997. Substantially lower infection level was found by Pawełczyk and Siński [22] in ticks collected in 1999 and 2000 in the Mazurian Lake District: 6.2% and 2.6%, respectively. On the other hand, exceptionally high prevalence values of *I. ricinus*, amounting to 24.5% (within 1994-1995) were found in Wielkopolska by Jenek and Głazaczow [10], while those found within 1997-1998 by Nowosad *et al.* [20] reached 22.6%.

In the present study a higher infection level of the adults was observed compared to nymphs (Tab. 1), which is consistent with the results obtained by the other authors. Also, a higher infection rate of the adults was recorded by Hubalek *et al.* [8], Hubalek *et al.* [7] and Hubalek and Halouzka [6] in the Czech Republic and in wooded areas of 16 European countries by Gray *et al.* [5]. Higher prevalence values of adult ticks with the spirochetes were also observed by Gilot *et al.* [4] in France, by Mejlon and Jaenson [14] - in southern Sweden, by Junttila *et al.* [11] in Finland, and by Stünzner *et al.* [29] in Austria. Similar differences in infection levels of individual developmental stages were stated in the course of some studies carried out in Poland. Wegner *et al.* [34] detected *B. burgdorferi* in 7.5% of nymphs and 18.8 % of the adults, collected in the wooded areas of Olsztyn. A study by the same authors within Gdańsk-Gdynia-Sopot agglomeration also demonstrated a lower level of infection of nymphs (8.2%) compared to females (14.9%) and males (18.9%). Stańczak *et al.* [28], studying infection levels of ticks in forests of 8 provinces of Poland, observed that the nymphs were less infected (6.2%) than females (14.9%) and males (12.4%). Similar proportions in prevalence values of *I. ricinus* obtained Jenek and Głazaczow [10] in their studies carried out within Wielkopolska (nymphs: 8.3%; females: 28.8%; and males: 22%). Some authors, however, observed a similar [25] or even lower [15, 35] infection of nymphs compared to the adults. Differences in infection frequency of individual developmental stages of *I. ricinus* in the papers of the cited authors might have resulted from influence of various ecological factors within the areas studied. In a final conclusion however, it has to be emphasized that nymphs, constituting a decisive majority in populations of *I. ricinus*, are more important in the transmission of the pathogens than the adult forms. Moreover, because of their smaller size, they are more difficult to detect while feeding on blood, which increases the risk of infection.

It is evident form the statistical analysis performed that there is a relationship between the quantity of *Ixodes ricinus* population and its infection level with the spirochetes, although such a correlation was more distinct and statistically significant only in 2000.

On the other hand, Ročkiene [24] attempted to determine a possible correlation between the frequency of cases of Lyme borreliosis and the seasonal activity of ticks and their infection level. According to this author, the rapid increase in the number of cases of borreliosis in Lithuania from 1991–1995 was related to growing numbers of ticks and increasing prevalence of *B. burgdorferi* in ticks. The results of the study have demonstrated that the frequency

of borreliosis cases coincided with seasonal increase of tick activity, and that was particularly high in autumn when many people visited forests.

It is possible that in the years to come, the percentage if infected ticks will not change in the wooded areas of Szczecin. Usage of chemical compounds and even a harsher winter may have only small effect on limiting the numbers of ticks, although without changing their infection level with spirochetes. The study by Matushchenko *et al.* [13] carried out in the forests of western Siberia revealed such high levels of infection of *I. persulcatus* as 58.3% in the area of Novosibirsk. This is a clear sign that low temperatures do not have a decisive impact on persistence of spirochetes in tick populations.

CONCLUSIONS

- Possibility of contacts between humans and ticks in the wooded areas of Szczecin create a high risk of acquiring Lyme borreliosis.
- Nymphs of *Ixodes ricinus* play a principal role in epidemiology of Lyme borreliosis.
- The highest probability of contacts between people and ticks in the areas studied has been determined for the months of the tick's highest activity i.e., June and August.

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