

BORRELIA BURGDORFERI SENSU LATO IN THE IXODES RICINUS TICKS IN SOUTHERN POLANDBranislav Peťko¹, Krzysztof Siuda², Michal Stanko³, Gabriela Tresová¹, Grzegorz Karbowski⁴, Jana Fričová³¹Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovakia²Department of Biology and Parasitology, Silesian Medical Academy, Katowice, Poland³Institute of Zoology, Slovak Academy of Sciences, Košice, Slovakia⁴W. Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

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Abstract: In May 1996, 164 pooled and single samples containing 913 larvae, nymphs and adults of *Ixodes ricinus* from urban, suburban and wooded biotopes of southern Poland were examined for the presence of *Borrelia burgdorferi* sensu lato. Ticks were collected by dragging a flag over the vegetation and *B. burgdorferi* was detected in pooled tick samples by darkfield microscopy or isolated by cultivation in BSK-II medium. Minimum infection rates of nymphs, males and females in the area were 8.2, 9.8 and 10.3%, respectively. Infection of adult *I. ricinus* ticks on 10 localities fluctuated from 4.0 to 15.0% and over 10% of adult ticks in forests and in some urban and suburban parks of the city Katowice were infected. Five *Borrelia* strains were isolated from the adult *I. ricinus* ticks. Relative density of ticks ranged from 20-65 ticks per one collecting hour and from 3.3-10.8 ticks per 100 m², respectively. Frequent occurrence of ticks was also observed in urban parks and residential districts of the city Katowice. The results refer to the risk of human and animal infections by borreliae in the areas of big cities in southern Poland.

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INTRODUCTION

Since its discovery in 1981, *Borrelia burgdorferi* sensu lato [3], etiological agent of Lyme borreliosis (LB), has been the subject of many epidemiological and epizootiological studies. This polysystemic disease of humans and animals has been recently regarded as the most common human tick-borne disease in the Holarctic. In natural foci, LB is transmitted primarily by blood-sucking arthropods, especially by ticks of the genus *Ixodes*: *I. ricinus* (L) in Europe, *I. persulcatus* Schulze in Eurasia, *I.*

pacificus Cooley et Kohls and *I. scapularis* Say in North America [1, 7, 23]. Infected ticks frequently occur in peripheral and central town parks in the territories of large cities in Central Europe. Therefore, we were interested in the occurrence and overinfestation of ticks by the agent of this dangerous zoonosis in the area of southern Poland with high concentration of large cities.

Little is known about the prevalence of *Borrelia* spirochetes in ticks in Poland. The most complete data was reported from the northeast provinces by the Institute of Maritime and Tropical Medicine in Gdynia [6, 24, 25, 26].



Figure 1. The locations of tick collection in South Poland: A) In Katowice and vicinity, B) In the northwest region of Tarnów province.

Ticks of the species *I. ricinus* and *Dermacentor reticulatus* were examined. Most of positive results were obtained from *I. ricinus*; the spirochetes *Borrelia* sp. have been observed recently also in *D. reticulatus* (Wegner, personal information).

Moreover, Siński *et al.* [20] published the results of a study on the *Borrelia burgdorferi* infection of ticks in Urwitał in Masuria Region and several places in the southeast Poland (including Katowice and Szczyglice near Kraków). Tylewska - Wierzbanowska *et al.* [22] examined ticks collected from vegetation, humans and animals in different provinces all over Poland. *I. ricinus* and *D.*

reticulatus mostly and occasionally *Argas reflexus* were examined in the above cited works. Positive results were obtained only for *I. ricinus*.

The occurrence of *Borrelia burgdorferi* in ticks in the closely neighbouring area of the Carpathians in Slovakia was studied by Kmety *et al.* [13], Prokopčáková *et al.* [19], Peřko *et al.* [15] and in the bordering area of the West Carpathians in Moravia by Hubálek *et al.* [9, 10] and Chmela [4].

This paper presents the studies on the occurrence of Lyme borreliosis agent in ticks in the areas of large cities in southern Poland over the year 1996.

MATERIAL AND METHODS

Studied area and collection of ticks. The density of tick populations and their overinfestation with LB agent, spirocheta *Borrelia burgdorferi* sensu lato, was studied in ten localities of southern Poland in May 1996 (Fig. 1). Ticks were collected by dragging a light woolen flag (60 × 90 cm) over the plant and bush vegetation. The collections were carried out primarily in the urban and suburban biotopes of the city Katowice (Fig. 2), in the places most frequently visited by people, e.g. in parks, sporting areas and peripheral villa districts. Some ticks were collected in wooded areas at places with low intensity of anthropogenic damaging (Chobot, Wał Ruda - Fig. 3). The area dragged with one flag in one hour measured approximately 600 m² in pastures and forests with shrubs, 800 m² in meadows and parks. Ticks collected from the particular localities were placed in test tubes with a few blades of grass and preserved at 4°C until investigation. The relative density of active ticks was expressed as the number of ticks collected with one flag per one hour, as well as a number of ticks per 100 m² (Tab. 1).

Table 1. Material of *Ixodes ricinus* ticks collected in southern Poland in May 1996.

Locality	Date	No. of ticks				Time dragged ^a	Area dragged ^b	RD ^c No./ hour
		males	females	nymphs	total			
1	May 24	45	55	3	103	3	2400	34
2	May 24	71	56	5	132	3	1800	44
3	May 29	37	36	52 ^d	125	3	1800	40
4	May 30	52	47	20	119	3	2400	40
5	May 30	31	39	5	75	3	2400	25
6	May 30	32	41	27	100	2.25	1350	44
7	May 30	30	43	10	83	2.25	1350	39
8	May 25	13	7	-	20	1	600	20
9	May 25	35	30	-	65	1	600	65
10	May 25	39	52	-	91	2	1200	46
Total		385	406	122 ^d	913	23.50	15900	40.3

^a - ... ^b - ... ^c - ... ^d - ...

Table 2. *Borrelia* species in *Ixodes ricinus* ticks collected in southern Poland.

Stage	Pool samples				Single samples			
	No. of			Infection rate (%) ^a	No. of		Infection rate (%)	
	ticks	pools	positive pools		ticks	positive		
Larvae (n = 6)	5	1	0	0	1	0	0	
Nymphs (n = 116)	110	22	9 (40.9%)	8.2	6	1	16.7	
Females (n = 406)	360	72	37 (51.4%)	10.3	46	13	28.3	
Males (n = 385)	345	69	34 (49.3%)	9.8	40	5	12.5	

^a calculated minimum infection rate.

Localities:

1. Mikołów near Katowice, in the “Barbara” coal mine area, meadow with mixed shrubs and trees.
2. Katowice, Ligota, oak-hornbeam forest and surrounding pastures with shrubs and dewberry.
3. Katowice, Kokociniec, forest and pastures with shrubs near railway.
4. Katowice, Wojewódzki Park Kultury i Wypoczynku Gen. J. Ziętka, park in surroundings of tennis-court.
5. Katowice, Park Tadeusza Kościuszki, old park with local occurrence of shrubs.
6. Katowice, Ochojec, forest near railway and residential district.
7. Katowice, Murcki, forest in surroundings of sports area.
8. Chobot (Kraków province), Niepołomicka Forest, old oak-hornbeam forest.
9. Borzęcin (Tarnów province), pastures with mixed shrubs and trees.
10. Wał Ruda (Tarnów province), pastures and meadows near forest with predominance of oaks and pines.

Investigation of ticks. Nymphs, males and females were investigated in pools of 5 specimens each. Moreover, some specimens were examined individually. Ticks were examined for the presence of *Borrelia* spp. by dark field microscopy, according to Wilske *et al.* [27] and by cultivation according to Preac-Mursic *et al.* [18] and Tresová *et al.* [21]. The samples were examined with a Nikon microscope at 400× magnification. Minimum infection rate of pools was calculated according to Kahl *et al.* [12].

A total of 164 pooled samples containing 820 specimens of *I. ricinus* were examined for the presence of *Borrelia burgdorferi* sensu lato. Another 93 ticks were investigated individually (Tab. 2).

RESULTS

In 1996, 913 larvae, nymphs and adult ticks *I. ricinus* were collected during a 23.5-hour vegetation flagging in southern Poland. Their relative density in 10 examined localities ranged between 20–65 ticks per one flag in one hour, or 2.8–12.3 ticks per 100 m², respectively (Tab. 1). Higher relative densities were observed on pastures in a

Table 3. Examination of pooled samples of *Ixodes ricinus* adults for *Borrelia* species in the localities of southern Poland.

Locality	No. of pools ^a		Positive pools ^b		Infection rate (%) ^c		Isolated ^d <i>Borrelia</i> strains
	Males	Females	Males	Females	Males	Females	
1	9	11	8	7	17.8	12.7	P153
2	12	9	6	7	10	15.5	
3	7	7	1	5	2.9	14.3	
4	8	7	2	1	5	2.9	P210
5	6	7	4	4	13.3	11.4	P175
6	6	8	0	5	0	12.5	
7	6	8	3	2	10	5	
8	2	1	0	0	0	0	
9	7	6	6	2	17.1	6.9	P137, P142
10	6	8	4	4	13.3	10	

^apool - 5 specimens, ^bdark field examination, ^ccalculated minimum infection rate, ^dcultivation.

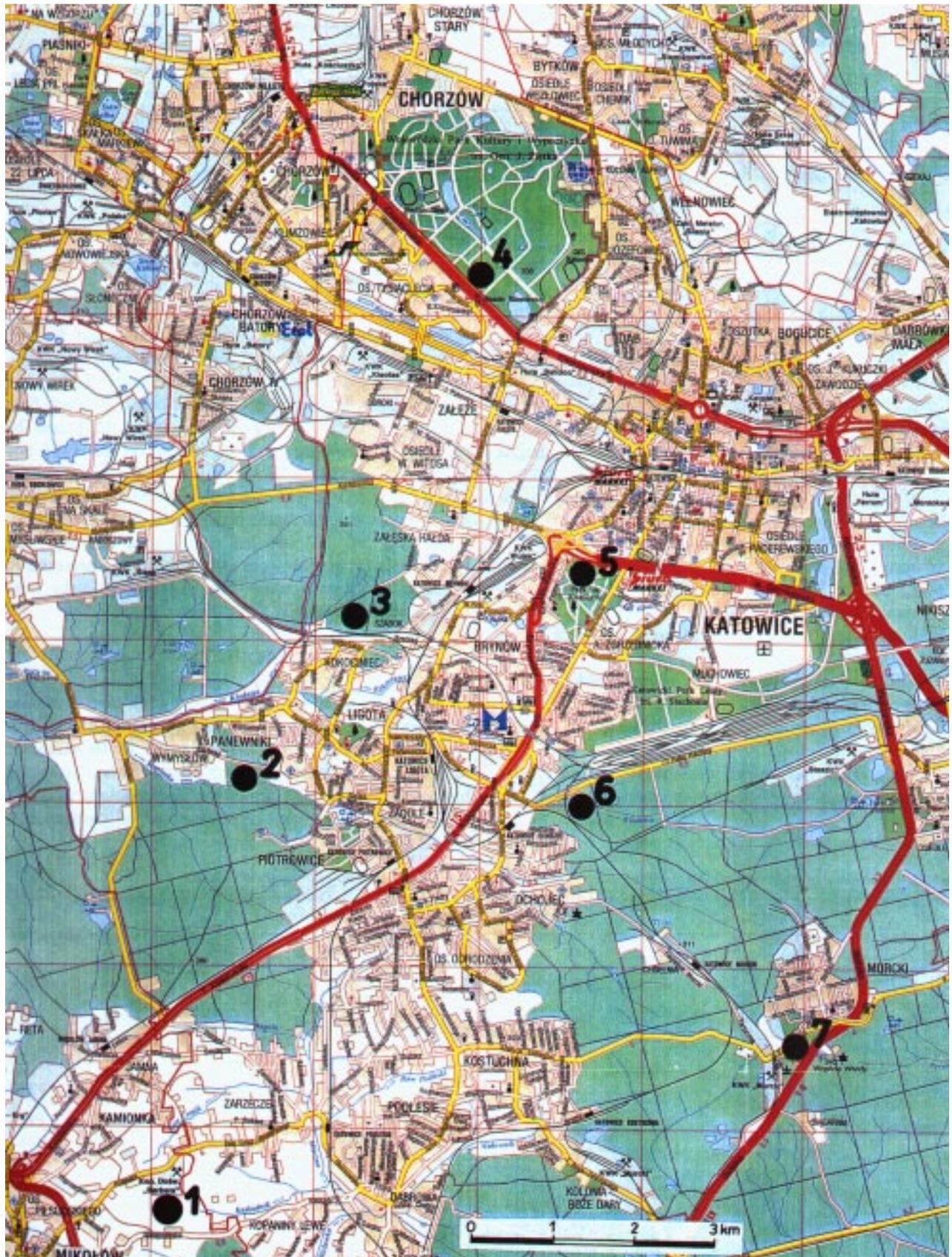


Figure 2. Tick collection localities in Katowice and vicinity: 1) Mikołów near Katowice, 2) Katowice – Ligota, 3) Katowice – Kokociniec, 4) Katowice – Wojewódzki Park Kultury i Wypoczynku Gen. J. Zięćka, 5) Katowice – Park Tadeusza Kościuszki, 6) Katowice – Ochojec, 7) Katowice – Murcki.

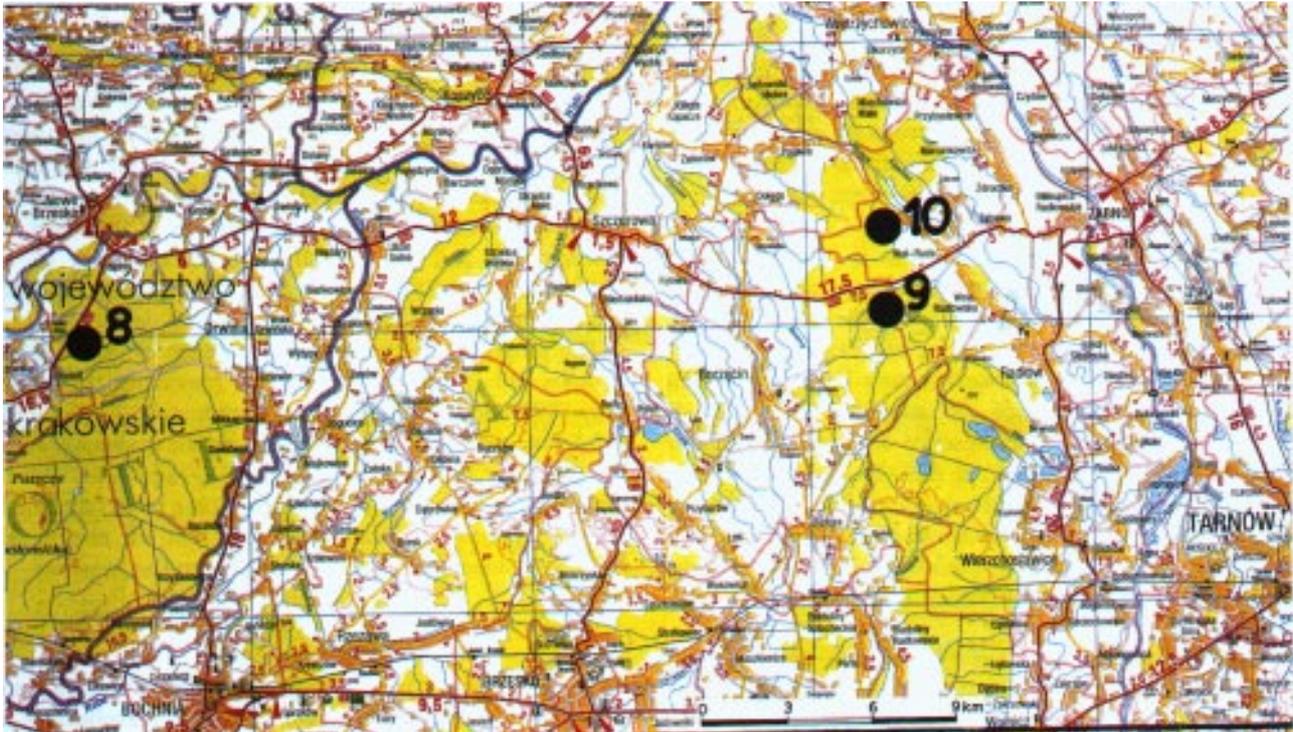


Figure 3. Tick collection localities in the northwest region of Tarnów province: 8) Chobot, 9) Borzęcin, 10) Wał Ruda

wooded area (loc. 9 and 10). Relatively high densities were also recorded in some urban and suburban parks and residential districts in the city Katowice (loc. 2, 3, 4, 6 and 7).

Moving spirochetes, morphologically resembling bacteria from *Borrelia* cultures, were observed in 49.3% of male and 51.4% of female *I. ricinus* pools. The lower values for nymphs (40.9%) were statistically insignificant ($t = 0.690$ and $t = 0.866$, respectively). Minimum infection

rate of *I. ricinus* nymphs, males and females was balanced (8.2–10.3%) in the entire studied area. Individually investigated ticks showed the proportion of infected females to be more than twice as high as for males. Single samples of larvae and nymphs were not evaluated because of a too small set (Tab. 2).

Borreliae were found in ticks from 9 localities (Tab. 3). The absence of infected ticks on locality 8 is probably due to the small number of examined specimens. Minimum infection rate of *I. ricinus* adult ticks at studied localities fluctuated from 4.0–15.0% without correlation with relative density of ticks (Fig. 4). A relative high infestation was observed in some suburban meadows and forests of the cities Katowice and Mikołów (loc. 1, 2), as well as in the urban park (loc. 5), which was higher than that in wooded rural areas (loc. 9 and 10).

Minimum infection rate of males and females at studied localities showed differences. At localities 2, 3 and 6, females were infected more frequently, at other localities it was males. Five strains of *Borrelia* spp. were isolated from tick pooled samples at localities 1, 4, 5 and 9. The strain marked as P142 comes from males, the others from females (Tab. 3). Immunochemical investigations of the isolated strains are published in other paper [21].

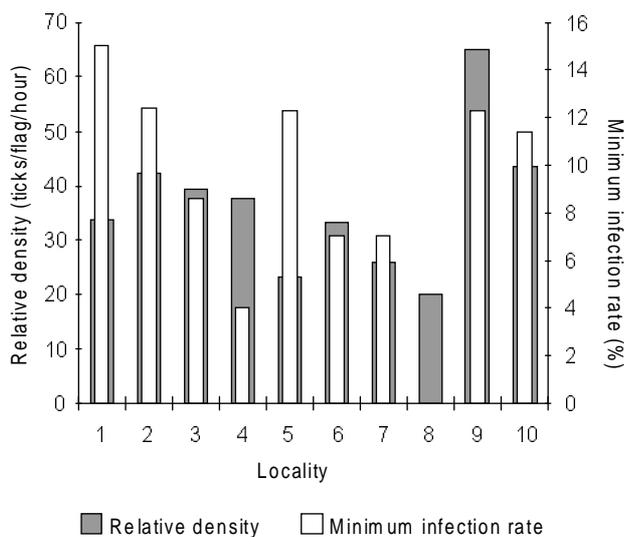


Figure 4. Relative density and minimum infection rate of *Ixodes ricinus* adults with *Borrelia* species in southern Poland.

DISCUSSION

Relatively high densities of ticks were found in May 1996 in the central and peripheral parks of the city of Katowice. Finding of ticks in central parks of large cities

(parks, gardens etc.) is a common phenomenon in the Central European region [2]. In the urban parks of Prague, Āerný and Daniel [5] ascertained 23.5–139.5 nymphs and adults per flag in one hour of collecting, with variable density during a year and also between different years. In a suburban park of the city Kořice, from 9–212 nymphs and adults of *I. ricinus* (flag/hour) were collected at 8 localities [15].

The occurrence of ticks in towns is connected with establishing new parks in original forest areas or with building new residential districts on the peripheries of towns close to forests. Persistence of ticks is determined by their possibility to accomplish their life cycle, which depends on the presence of suitable hosts for all developmental stages. The presence of hosts is important for adult ticks. In Central Europe these hosts are represented mainly by squirrels (*Sciurus vulgaris*), both species of hedgehogs (*Erinaceus europaeus* and *E. concolor*) and local stray dogs and cats. A sporadic occurrence of ticks in isolated parks can be attributed to the transmission by birds.

The occurrence of ticks has a mosaic-like character and their infection with borreliae at different places of an area with the presence of LB is highly variable, depending on the place and time of investigation [28]. Cases with seasonal alteration were observed in local populations of ticks [9, 13, 15].

Long-term observations under Central European conditions showed an impressive local fluctuation in the infestation of ticks in the individual years of study: 6.5–11.4% [10]; 1.9–22.0% [16]; 0.9–18.6% [17]; 2.1–41.7% [15]. These variations in tick infestation are most likely reflecting the periodical occurrence of borreliae in ticks, which was pointed out in Slovakia by Kmety *et al.* [13]. In the Carpathian region of Slovakia this appears as a 4–5 year cycle, with the last minimum recorded in 1994 and maximum in 1992–1993 [15]. In comparing the values of the LB prevalence at different places of this area, the phase of epizootiological cycle should also be considered.

The high variability of *Borrelia* infection in ticks at individual developmental stages is probably associated with the periodicity of LB. Adult ticks become generally more frequently infected than nymphs, females more frequently than males. Kahl *et al.* [12] reported a minimum 2.5% infection rate of nymphs, 5.3% of males and 10.2% of females from the territory of Berlin in 1986. Percentage of ticks infected by spirochetes of *Borrelia* spp. examined in Poland varied. In Olsztyn province it has ranged from 2.9–35.7% (average 11.5%) [25], in Urwitałt in Masuria region 3.5% and in examined localities in southeast Poland from 13–58.3% (in Szczyglice near Kraków 19.2%) [20]. Among *I. ricinus* specimens from different parts of Poland tested by Tylewska-Wierzbanowska *et al.* [22] only 0.75% of ticks were infected by *B. burgdorferi*. In southern Germany, Wilske *et al.* [27] found an average 1% infection in larvae, 10% in nymphs and 20% in adult ticks, with no difference observed between the infection rate in males and females. Nymphs are less frequently infected than adult

ticks [20, 25, 26], as was observed at several localities in 1992 [8, 15]. A variation in the prevalence of infected adults is generally preceded by the change in the infection rate of nymphs in the previous year. In both mentioned cases, an increased prevalence of infected adults occurred during the next year or the next two years. These differences may reflect the presence of *Borrelia* donors (animals at the stage of bacteraemia) and of naturally immunized hosts of *I. ricinus*, which may cause either the increase in the occurrence of borreliae [11] or their loss in ticks [14].

The proportion between the prevalence of borreliae in nymphs and adults and the absolute prevalence values in May 1996 may serve as a basis for estimating the phase of epizootiological cycle and for prognosing the epidemiological situation for LB in the region. A significant local variability of infected tick prevalence on a relatively small territory points out the wide spectrum of ecological factors influencing the occurrence of borreliae in nature.

CONCLUSION

The preliminary results indicate the hazard of human infection elicited by the agent of Lyme borreliosis - a grave natural focal zoonosis in the urban and suburban verdure of large cities in southern Poland, which should not be underestimated. Relatively high prevalence of infected ticks and the balanced proportion of nymph and adults infestation with borreliae in southern Poland suggests the culmination of cycle and its possible persistence in the year 1997.

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