POPULATION DYNAMICS AND *BORRELIA BURGDORFERI* INFECTION RATE OF *IXODES RICINUS* TICKS IN THE BELGRADE AREA

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To obtain better understanding of epidemiological and epizootiological agents inside a specific region, investigations of ticks in the Belgrade area, an endemic focus of Lyme disease, were carried out during 2002. Green areas of four localities were investigated: Ada Ciganlija, Košutnjak, Miljakovačka forest and Avala. The research involved: relative abundance, population dynamics and detection of Borrelia burgdorferi in Ixodes ricinus ticks. A total of 2704 ticks were collected. The greatest number of ticks were recorded on Avala (1265), followed by Miljakovačka forest (614), Košutnjak (541) and Ada Ciganlija (284). Maximal abundance of ticks was recorded in mid-April at Ada Ciganlija, but during May in other localities. A total of 530 ticks of the species Ixodes ricinus (from all localities) was examined by darkfield microscopy for Borreliae. Out of this number, 125 (23.58 %) were infected with Borrelia burgdorferi. The proportions of infected ticks were 20.34 % in nymphs and 23.99 % in adults. Infection rates by localities were: Avala - 18.75 %, Miljakovačka forest – 20.21 %, Ada Ciganlija – 29.31 % and Košutnjak - 31.88 %. There were statistically significant differences in the infection rates between localities (p<0.05).

Key words: Ixodes ricinus, population dynamics, Borrelia burgdorferi

INTRODUCTION

Lyme borreliosis, caused by the spirochaete *Borrelia burgdorferi* s. l.*,* is the most prevalent arthropod-transmitted zoonosis in Europe. Concerning domestic animals, Lyme borreliosis has been reported in dogs, cats, horses, cattle and sheep (Bushmich 1994).

Research carried out on Lyme disease in Serbia so far pointed to the species *Ixodes ricinus* L. (*Acari: Ixodidae*) as a source, vector and reservoir in the epizootic process of *Borrelia burgdorferi*. The species *Ixodes ricinus* is predominant in material originating from Serbia. Moreover, this species is one of the most widely distributed in Serbia and Montenegro (Petrović and Milutinović, 1993). In Serbia the dynamics of *Ixodes ricinus* shows two phases of seasonal

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fluctuation: spring and autumn (Milutinović and Radulović, 2002). The turn of seasons may influence disease prevalence and result in a periodical occurrence.

The first cases of Lyme disease in the Belgrade area were diagnosed in 1987 (Dmitrović *et al*. 1989). *Borrelia burgdorferi* was first isolated from ticks collected in the Belgrade area in 1990 (Dmitrović 1996). Concerning borreliae in ticks collected in parks of Belgrade, Stajković *et al*. (1996) reported that 21% of *Ixodes ricinus* individuals in 1994 and 26% of *Ixodes ricinus* in 1995 were positive for *Borreliae*. Otherwise, the overall rate of infection was 31.1% in adults and 13.6% in nymphs in three different natural localities in the Belgrade area (Avala, Košutnjak and Zvezdara) in 1998 (Milutinović, 2000).

The objectives of this study were to monitor population dynamics of *Ixodes ricinus* ticks and to compare the prevalence of borreliae for nymphal and adult *Ixodes ricinus* in four natural foci of Lyme borreliosis in the district of Belgrade. Study areas represent important recreational sites with high frequency of presence of people and domestic animals (mostly pet dogs).

MATERIAL AND METHODS

Ticks were collected by dragging white flannel "flags" (1 m^2) over vegetation from March to July 2002 in four localities of the Belgrade area, namely: Ada Ciganlija, Košutnjak, Miljakovačka forest and Avala. Unfavourable environmental conditions (temperature, relative air humidity and precipitation) caused a decrease of abundance of the autumn tick population. There are differences in the type of vegetation in the investigated localities. While Avala and Miljakovačka forest represent typically wooded localities, Ada Ciganlija and Košutnjak are a mixture of parklands and small wooded complexes.

Sampling was made once weekly during daylight hours, mainly between 10 a.m. and 4 p.m. Relative tick abundance was estimated by determining the average number of ticks per person per hour. All collected ticks were maintained in moistured plastic tubes at +5 ^{o}C until assay.

Each tick was examined individually: the abdomen was dissected in its ventral part, the midgut and other internal organs excised, suspended in a drop of physiological saline on a microscopic slide, squashed with a coverslip, and examined for borreliae using dark-field illumination and 400 × magnification (Mejlon, 2000).

Differences in the percent of infected ticks between the four localities were analysed using the chi-square test (2×4 frequency table). The level of statistical significance was less than 5 %.

RESULTS AND DISCUSSION

In the period March – July 2002 a total of 2704 *Ixodes ricinus* ticks were collected in four localities of the Belgrade area. Adults were more numerous accounting for 2399 or 88.72 % of the total number of collected ticks, in contrast to

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305 nymphs (11.28 %). Moreover, males were prevalent (1250 / 46.23 %) in contrast to females (1149 / 42.49 %).

Regarding abundance by localities, the greatest number of ticks were recorded on Avala (1265 / 46.78 %), followed by Miljakovačka forest (614 / 22.71%), Košutnjak (541 / 20.01 %) and Ada Ciganlija (284 / 10.50 %). The mean relative abundance (the average number of ticks per person per hour) by localities were: Avala – 45.18**,** Miljakovačka forest - 23.62, Košutnjak - 11.76, Ada Ciganlija - 6.45 (Table 1).

Thus, the mean relative abundance of ticks in Miljakovačka forest was 2 times greater than the mean relative abundance at Košutnjak and about 3.7 times greater than the mean relative abundance on Ada Ciganlija. The mean relative abundance of ticks on Avala was 3.8 and 7 times greater in contrast to the mean relative abundance at Košutnjak and Ada Ciganlija, respectively. Therefore, the abundance of ticks in wooded localities (Miljakovačka forest and Avala) was significantly greater than in parkland localities (Ada Ciganlija and Košutnjak). In the parklands of Ada Ciganlija and Košutnjak, anthropogenic factors influence the abundance of ticks, both directly (by grass cutting) and indirectly (by changing microclimate conditions). A similar findings was obtained by Stajković and Radulović (1999).

The population dynamics of ticks was monitored from March to July 2002. Maximal abundance of ticks was recorded in mid-April at Ada Ciganlija, but in other localities during May (Košutnjak at the beginning, Avala in mid-May and Miljakovačka forest at the end of the month) (Figure 1).
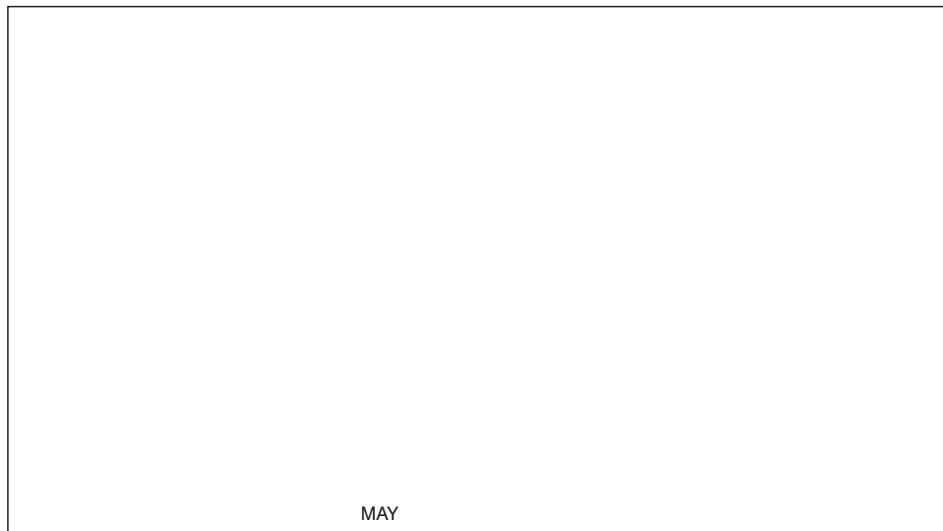

MAY

Figure 1. Population dynamics of ticks.

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Table 1. Abundance of *Ixodes ricinus* ticks at four localities of the Belgrade area in 2002.

	Ada Ciganlija			Košutnjak			Miljakovačka forest			Avala			Total		
	No	f h	No/f h	No	f h	No/f h	No	f h	No/f h	No	f h	No/f h	No	f h	No/f h
March I	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
March II	9	4	2.25	9	4	2.25	–	–	–	–	–	–	20	8	2.50
April I	69	6	11.50	55	6	9.17	20	4	5.00	30	4	7.50	187	20	9.35
April II	81	6	13.50	107	6	17.83	71	4	17.75	191	4	47.75	460	20	23.00
May I	45	6	7.50	159	6	26.50	145	4	36.25	376	4	94.00	725	20	36.25
May II	34	6	5.67	117	6	19.50	211	4	52.75	327	4	81.75	689	20	34.45
Jun I	21	6	3.50	49	6	8.17	98	4	24.50	219	4	54.75	411	20	20.55
Jun II	17	6	2.83	23	4	5.75	51	4	12.75	72	4	18.00	163	18	9.06
July I	8	4	2.00	9	4	2.25	18	2	9.00	31	2	15.50	66	12	5.50
July II	–	–	-	13	4	3.25	–	–	–	19	2	9.50	32	6	5.33
Total	284	44	6.45	541	46	11.76	614	26	23.62	1265	28	45.18	2753	144	19.12

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A total of 530 ticks of the species *Ixodes ricinus* (from all localities) were examined by dark-field microscopy for borreliae. Out of this number, 125 (23.58 %) specimens were infected with *Borrelia burgdorferi*. The proportions of infected ticks by localities were as follows: Avala – 18.75 %, Miljakovačka forest – 20.21 %, Ada Ciganlija – 29.31 % and Košutnjak – 31.88 %. The overall mean infection rate was 27.35 % in male, 20.35 % in female, 23.99 % in adults and 20.34 % in nymphs (Table 2).

Table 2. Borreliae in male, female and nymphs of *Ixodes ricinus* ticks collected in four localities of the Belgrade area in 2002.

Locality	Males		Females		Nymphs		Total	
Ada Ciganlija	8/25	32.00%	7/26	26.92%	2/7	28.57%	17/57	29.31%
Košutnjak	26/68	38.24%	14/57	24.56%	4/13	30.77%	44/138	31.88%
Miljakovačka forest	10/42	23.81%	7/40	17.50%	2/12	16.67%	19/94	20.21%
Avala	23/110	20.91%	18/103	17.48%	4/27	14.81%	45/240	18.75%
Total	67/245	27.35%	46/226	20.35%	12/59	20.34%	125/530	23.58%

There was a significant difference in the prevalence of *Borrelia burgdorferi* in ticks in other European countries. The overall rate of infection by borreliae of the *Ixodes ricinus* ticks in the studies of Kmety *et al*. (1986) and Hubálek *et al.* (1990) in south Moravia and western Slovakia was about 8 %. Štepánová-Tresová *et al*. (1999) reported about 4.8 % infected ticks in 1997 and 11.04 % infected ticks in 1998 in southern Bohemia. The overall infection rate in Brno park Pisarky was 9.3 % in 1996 and 1997 (Žákovská 1999). Genchi *et al*. (1994) reported a high prevalence of *Borrelia burgdorferi* (15 – 20 %) in ticks from Laghi di Lamar, Italy. For 23 provinces of Sweden, Gustafson *et al*. (1995) pointed out that prevalence of infection in adult *Ixodes ricinus* ranged from 3 % to 23 %. Moreover, their study showed that *Borrelia burgdorferi* sensu lato was present throughout the distributional area of *Ixodes ricinus* in Sweden.

In spite of the small distance between the four investigated localities in our research, which ranged from 10 to 20 km, there were statistically significant differences in *Borrelia burgdorferi* infection rates of *Ixodes ricinus* ticks (χ^2=9.9988; df=3; p<0.05). Here, it seems appropriate to mention Hubálek *et al*. (1991) who found for two close areas of southern Moravia that the proportions of infected ticks were 3.8% in nymphs and 10.6% in adults of area A, while they were 29.1% in nymphs and 35.9% in adults of area B.

The obtained results point to a negative relationship between tick abundance and tick infection rates in our research (Table 3). A negative relationship between those parameters was also reported by Gray *et al*. (1999).

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Table 3. Relationship between tick infection rates and tick relative abundance from four localities of the Belgrade area in 2002.

Locality	Infection rate	Mean relative abundance (No/fh)
Avala	18.75 %	45.18
Miljakovačka forest	20.21 %	23.62
Ada Ciganlija	29.31 %	6.45
Košutnjak	31.88 %	11.76

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DINAMIKA POPULACIJA I INFICIRANOST KRPELJA *IXODES RICINUS* SA *BORRELIA BURGDORFERI* NA TERITORIJI BEOGRADA

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SADRŽAJ

U periodu mart-jul 2002. godine praćena je dinamika populacije i inficiranost krpelja *Ixodes ricinus* sa *Borrelia burgdorferi*. Istraživanja su obavljena na četiri lokaliteta područja Beograda: Ada Ciganlija, Košutnjak, Miljakovačka šuma i Avala. Maksimalna brojnost krpelja zabeležena je sredinom aprila na Adi Ciganliji i tokom maja u Košutnjaku, Miljakovačkoj šumi i na Avali. Ustanovljene su statistički značajne razlike u inficiranosti krpelja sa *Borrelia* burgdorferi po lokalitetima ($\chi^2=9.9988$; df=3; p<0.05).