Arch. Biol. Sci., Belgrade, 58 (3), 183-186, 2006.

SEASONAL DISTRIBUTION OF BORRELIAE IN *IXODES RICINUS* TICKS IN THE BELGRADE REGION, SERBIA

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Abstract - Green areas at four localities in the Belgrade region (Ada Ciganlija, Košutnjak, Miljakovac Forest, and Mt. Avala) were investigated in 2004. The aim of the research was to clarify the faunistic composition, relative abundance, and population dynamics of ticks, as well as the seasonal distribution of *Borrelia burgdorferi sensu lato (sl)* in *Ixodes ricinus*. Two species of ticks were detected: *Ixodes ricinus* and *Dermacentor reticulates*. Relative abundance analysis revealed that the species *Ixodes ricinus* was predominant (97.41 %). Out of 942 *Ixodes ricinus* ticks, 188 (19.96 %) were infected with *Borrelia burgdorferi sl*. The infection rate of adults by localities ranged from 19.16% to 30.99% (Mt. Avala and Ada Ciganlija, respectively).

Key words: Ticks, relative abundance, population dynamics, Ixodes ricinus, Borrelia burgdorferi, Serbia

UDC 595.42:616.993(497.111)

INTRODUCTION

In research on ticks as reservoirs and vectors of the causative agents of disease, it is of great importance to ascertain elements of zoogeographic distribution, particularly the possible presence of species which might be indicators of a disease. As the ecology of each etiological agent of disease is reflected in the ecology of the host, the aim of research should be to clarify ecological factors auch as natural foci, infection agents, reservoirs, modes of transmission, nosogeography (the geography of infections and diseases), seasonal distribution, incidence/prevalence, vectors, and vector biology. It should also deal with prevention/control, isolation of infection agents, and their identification. Faunistic and ecological invectigations of ticks in the Belgrade region [(M i l u t i n o v i ć and Petrović 1993; Milutinović et al., 1996, 1997/8, 2002; Petrović and Milutinović (in press)] can contribute to drafting of more effective programs for control and eradication of these arthropods.

Among all European tick species, *Ixodes ricinus* is the most widely distributed. Its wide ecological amplitude and very aggressive and undiscriminating behavior during questing for a host make it one of the main transmitters of causative agents of infectious diseases among the human population.

Research carried out so far on Lyme disease in Europe and Serbia has pointed to the species *Ixodes ricinus* as a source, vector, and reservoir of *Borrelia burgdorferi* sensu lato (sl). The first cases of Lyme disease in the Belgrade region were diagnosed in 1987 (D m i t r o v i ć et al., 1989). *Borrelia burgdorferi sl* was first isolated from ticks collected in the Belgrade region in 1990 (D m i t - r o v i ć, 1996). The overall rate of infection was 31.1% in adults and 13.6% in nymphs at three different natural localities in the Belgrade region (Mt. Avala, Košutnjak and Zvezdara) in 1998 (M i l u t i n o v i ć, 2000).

The aim of this study was to clarify differences in abundance and population dynamics of ticks and ascertain seasonal distribution of borreliae in *Ixodes ricinus* ticks at four localities in the Belgrade region, Serbia.

MATERIAL AND METHODS

Ticks were collected by flagging vegetation at four localities in the region of Belgrade (Ada Ciganlija, Košutnjak, Miljakovac Forest, and Mt. Avala) in the period of March-November 2004. Evaluation of questing tick abundance was performed according to R a n d o l p h *et* Table 1. Population dynamics of Ixodes ricinus ticks.

November	No/ 00 m ²	0.00	00.0	0.00	0.00	00.0	0.00	0.67	0.33
	No	0)	0	0	0	0		
)						_
October	No/ 100 m ²	0.00	0.00	0.00	0.00	1.00	0.67	1.33	1.33
	No	0	0	0	0	m	7	4	4
September	$\frac{No}{100}$ m ²	0.33	1.00	0.00	0.67	1.67	1.33	2.67	1.00
	٥N	1	m	0	2	Ś	4	8	т
August	No/ 100 m ²	0.67	0.67	1.33	1.33	1.00	0.33	1.33	0.67
	No	2	7	4	4	3	-	4	2
July	$N_0/$ 100 m ²	1.33	0.00	1.67	0.67	7.00	1.33	7.00	4.67
	No	4	0	5	5	21	4	21	14
June	N_{0} / 100 m ²	8.33	1.67	8.33	6.00	14.00	7.67	13.33	30.67
	No	25	5	25	18	42	23	40	92
May	N_{O} / $100 m^2$	7.00	4.00	4.33	14.33	3.33	19.67	9.33	23.00
	No	21	12	13	43	10	59	28	69
March April	$\frac{N_{O}}{100}$ m ²	2.67	11.67	1.33	20.67	1.67	25.00	4.67	7.33
	No	8	35	4	62	5	75	14	22
	${}^{\mathrm{No}'}_{100 \mathrm{m}^2}$	0.67	4.67	0.33	5.33	0.00	4.67	1.00	2.33
	No	2	14	1	16	0	14	3	7
Locality Stage		ty Nymphs Adults		Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
		Ada Ciganlija		Košutnjak		Miljakovac Forest		Mt. Avala	

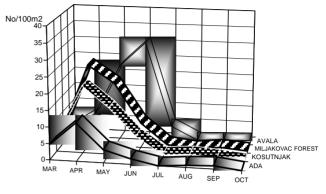


Fig. 1. See the text.

al. (2002). The ticks were examined by dark-field illumination and 400 x magnification for borreliae (M e j l o n, 2000).

Differences in tick infection prevalences between localities were analyzed by means of the χ^2 -test.

RESULTS AND DISCUSSION

In the period of March-November 2004, a total of 967 ticks were collected at four localities in the region of Belgrade (Ada Ciganlija, Košutnjak, Miljakovac Forest, and Mt. Avala). The greatest number of ticks were recorded at the Mt. Avala (338), followed by Miljakovac Forest (271), Košutnjak (199), and Ada Ciganlija (159). The number of ticks per 100 m² slightly varies between localities (Table 1).

Relative abundance analysis revealed that the species *Ixodes ricinus* was absolutely dominant (942/97.41%) in relation to *Dermacentor reticulatus* (25/2.59%). All specimens of the species *D. reticulatus* were collected at the Ada Ciganlija locality. P e t r o v i ć and M i l u t i n o v i ć (*in press*) indicate the presence of four tick species (*Ixodes ricinus*, *Rhipicephalus sanguineus*, *Dermacentor marginatus*, and *Dermacentor reticulatus*) and absolute dominance of the species *Ixodes ricinus* in green areas of the Belgrade region.

The population dynamics of ticks was monitored from March to November 2004. Maximal abundance of ticks was recorded at the Ada Ciganlija locality in mid-April, but at other localities during May (Košutnjak in the first decade, Miljakovac Forest in the second, and Mt. Avala in the third) (Fig. 1).

Unfavorable environmental conditions (tempera-

Locality	Nymphs		Females		Males		Adults	
Mt. Avala	14/124	11.29%	22/111	19.82%	19/103	18.45%	41/214	19.16%
Miljakovac Forest	11/89	12.36%	20/88	22.73%	22/94	23.40%	42/182	23.08%
Košutnjak	7/52	14.46%	23/77	29.87%	17/70	24.29%	40/147	27.21%
Ada Ciganlija	11/63	17.46%	9/34	26.47%	13/37	35.14%	22/71	30.99%
Total	43/328	13.11%	74/310	23.87%	71/304	23.36%	145/614	23.62%

Table 2. Distribution and frequency of occurence of borreliae in *Ixodes ricinus* ticks.

ture, relative air humidity, and precipitation) caused a decrease in abundance of the tick population in autumn. There are differences in the type of vegetation at the investigated localities. While Mt. Avala and Miljakovac Forest represent typical wooded localities, Ada Ciganlija and Košutnjak are a mixture of parklands and small wooded complexes. Logically, the abundance of ticks was significantly greater at the wooded localities (Miljakovac Forest and Mt. Avala) than at the parkland localities (Ada Ciganlija and Košutnjak). In the parklands of Ada Ciganlija and Košutnjak, the anthropogenic factor affect the abundance of ticks, both directly (by grass cutting) and indirectly (trough alteration of microclimatic conditions). Similar findings were reported by M i l u t i n o v i ć *et al.* (2004).

Maximal abundance of ticks in mid-April at the Ada Ciganlija locality is to some extent is a consequence of the presence the species *Dermacentor reticulatus*, whose maximal abundance occurs during this period in the given study area (M i l u t i n o v i ć *et al.*, 1998). copy for borreliae. Out of this number, 188 (19.96 %) were infected with *Borrelia burgdorferi sl*. The infection rate ranged from 19.16 % to 30.99 % (at the Mt. Avala and Ada Ciganlija localities, respectively) in adults and from 11.29% (Mt. Avala) to 17.46% (Ada Ciganlija) in nymphs. The overall mean infection rate was 23.87 % in males, 23.36 % in females, 23.62 % in adults, and 13.11 % in nymphs (Table 2).

Differences in seasonal distribution of borreliae in *Ixodes ricinus* ticks were not statistically significant (χ^2 -test, p<0.05), but there are statistically significant differences in infection rates between localities (χ^2 -test, p<0.05).

The results of these investigations with detailed study of the bionomy of ticks as vectors in the Belgrade region will contribute to a better understanding of the epidemiological and epizootiological situation and result in drafting of programs of prevention/ control/eradication.

Locality	Stage	March	April	May	June	July	AugNov.
Mt. Avala	Nymphs	0.00%	7.14%	10.71%	15.00%	9.52%	11.11%
Ivit. Avala	Adults	14.29%	18.18%	24.64%	16.30%	14.29%	20.00%
Miljakovac	Nymphs	0.00%	20.00%	20.00%	11.90%	9.52%	9.09%
Forest	Adults	21.43%	22.67%	27.12%	17.39%	25.00%	14.29%
Košutnjak	Nymphs	20.00%		7.69%	16.00%	11.11%	
Kosutijak	Adults	31.25%	30.65%	25.58%	16.67%	25.0	00%
Ada Ciganlija	Nymphs	20.00%		19.05%	16.00%	14.29%	
Ada Ciganiija	Adults	35.71%	31.43%	33.33%	20.00%	20.0	00%

Table 3. Seasonal distribution of borreliae in Ixodes ricinus.

A total of 942 ticks of the species *Ixodes ricinus* (from all localities) were examined by dark-field micros-

Acknowledgement - This research was supported by the Ministry of Science and Environment Protection of the Republic of Serbia (Projects 145002B and 143006B).

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СЕЗОНСКА ДИСТРИБУЦИЈА БОРЕЛИЈА У КРПЕЉИМА *IXODES RICINUS* НА ТЕРИТОРИЈИ БЕОГРАДА, СРБИЈА

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Зелене површине четири локалитета на подручју Београда обрађене су током 2004. године: Ада Циганлија, Кошутњак, Миљаковачка шума и Авала. Истраживања су обухватила: фаунистички састав, релативну бројност, динамику популација крпеља и сезонску дистрибуцију *Borrelia burgdorferi sensu lato (sl)* у крпељима *Ixodes ricinus*. Установљене су две врсте крпеља: *Ixodes ricinus* и *Dermacentor reticulates*. Анализа релативне бројности показује да је врста *Ixodes ricinus* била доминантна (97.41%). Од 942 крпеља *Ixodes ricinus*, 188 је било инфицирано са *Borrelia burg-dorferi sl*. Проценат заражених адулта по локалитетима се кретао од 19.16% на Авали до 30.99 % на Ади Циганлији.

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