



## Prevalence of *Borrelia burgdorferi sensu lato* in *Ixodes ricinus* ticks and assessment of entomological risk index at localities in Belgrade

### Prevalencija *Borrelia burgdorferi sensu lato* kod krpelja *Ixodes ricinus* i procena entomološkog indeksa rizika na lokalitetima Beograda

Milena Krstić\*†, Novica Stajković\*†, Srdjan Lazić\*†

\*Institute of Epidemiology, Sector for Preventive Medicine, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

#### Abstract

**Background/Aim.** The first case of human Lyme borreliosis (LB) in Serbia was recorded in 1987. The number of reported LB cases has increased in the past decade. The aim of this study was to estimate the density of *Ixodes ricinus* (*I. ricinus*) ticks, the prevalence of *Borrelia burgdorferi sensu lato* (*B. burgdorferi*) in them, and entomological risk index (ERI) at 19 Belgrade localities which were grouped into three categories (forests, park-forests, parks). The values of ERI were compared with the number of tick bites in humans. **Methods.** Ticks were collected monthly by using the flag hours method and the infection rate was determined by using dark field microscopy. The ERI value was calculated for each locality where the ticks were collected. The related data about tick bites was obtained from the patient protocol of the Institute of Epidemiology, Military Medical Academy, Belgrade. **Results.** The total number of collected ticks, the number of nymphs and the infection rates of the nymphs were significantly higher in forests ( $p < 0.05$ ) than park-forests and parks. Statistically, the ERI value was significantly higher in forests than parks of Belgrade ( $\chi^2 = 7.78, p < 0.01$ ). In March and July, the ERI value was also significantly higher in forests, than park-forests ( $p < 0.01$ ) and parks ( $p < 0.01$ ). May was the month with the highest ERI value in each ecological category (forests  $p < 0.05$ ; park-forests  $p < 0.01$ ; parks  $p < 0.001$ ). However, the number of tick bites in humans did not correlate with ERI values. **Conclusion.** The obtained results indicate that the risk of tick bite and human exposure to *B. burgdorferi sensu lato* is present at all selected localities in Belgrade. For a more comprehensive Lyme disease risk assessment the method of entomological risk index assessment should be combined with other methods, taking into consideration all tick stages and the behaviour and habits of people who may get infected *B. burgdorferi sensu lato*.

#### Key words:

ticks bites; ixodes; borrelia burgdorferi; lyme disease; prevalence; population density; health status indicators; serbia.

#### Apstrakt

**Uvod/Cilj.** Prvi slučaj lajmske borelioze (Lyme borreliosis, LB) u Srbiji zabeležen je 1987. Broj prikazanih bolesnika sa LB porastao je tokom poslednje dekade. Cilj ovog istraživanja bio je da se procene gustina krpelja *Ixodes ricinus* (*I. ricinus*), prevalencija *Borrelia burgdorferi* (*B. burgdorferi*) u njima i entomološki indeks rizika (ERI) na 19 beogradskih lokaliteta grupisanih u tri ekološke kategorije (šume, park-šume, parkovi). Vrednosti ERI upoređivane su sa brojem uboda krpelja kod ljudi. **Metode.** Krpelji su prikupljeni mesečno pomoću metode *flag* časa, a stopa infekcije određivana je u tamnom polju mikroskopa. ERI vrednost je izračunavana za svaki lokalitet na kome su sakupljeni krpelji. Podaci o ubodima krpelja stanovnika dobijeni su iz Protokola ispitanika za ubod krpelja Instituta za epidemiologiju Vojnomedicinske akademije u Beogradu. **Rezultati.** Ukupan broj prikupljenih krpelja, broj nimfi i stopa infekcije nimfi bili su značajno viši u šumama ( $p < 0,05$ ) nego u park-šumama i parkovima. Utvrdili smo statistički značajno višu vrednost ERI u šumama nego u parkovima Beograda ( $\chi^2 = 7,78, p < 0,01$ ). U martu i julu, značajno viša vrednost ERI ustanovljena je u šumama nego u park-šumama ( $p < 0,01$ ) i parkovima ( $p < 0,01$ ). Maj je bio mesec sa najvišim vrednostima ERI u svakoj ekološkoj kategoriji (šume  $p < 0,05$ ; park-šume  $p < 0,01$ ; parkovi  $p < 0,001$ ). Broj uboda krpelja kod ljudi nije bio u korelaciji sa vrednostima ERI. **Zaključak.** Dobijeni rezultati pokazuju da na svim odabranim lokalitetima Beograda postoji rizik od uboda krpelja i ekspozicije ljudi *B. burgdorferi*. Za sveobuhvatniju procenu rizika od lajmske bolesti, metodu procene ERI trebalo bi kombinovati sa drugim metodama, uzimajući u obzir sve stadijume krpelja, kao i ponašanje i navike ljudi, koji se mogu inficirati *B. burgdorferi sensu lato*.

#### Ključne reči:

krpelj, ubodi; ixodes; borrelia burgdorferi; lajmska bolest; prevalenca; populacija, gustina; zdravstveno stanje, indikatori; srbija.

## Introduction

The first case of human Lyme borreliosis (LB) in Serbia was recorded in 1987<sup>1</sup>. The number of reported LB cases has increased in the past decade. According to the reports from the Institute for Public Health of Serbia, a total of 3,860 patients were registered in the period 2002–2007, with the average annual incidence of 10.7/100 000. Between 2006 and 2008 more than 200 cases of LB were reported in Serbia. The majority of LB cases were found in Belgrade. In the period from 2000 to 2007, a total of 3,126 persons with tick bites were referred to the Institute of Epidemiology, Military Medical Academy in Belgrade<sup>2</sup>.

Due to the high number of LB cases in Belgrade, the Program of Prevention and Chemical Fighting of Ticks was implemented in 1994. The application of preventive measures (personal protection and landscape management) has priority in the Program, but according to some ecological and epidemiological criteria (an increasing number of tick bitten humans, an increasing incidence of LB, an increasing number of ticks and the infection rate of *B. burgdorferi sensu lato*), it is necessary to perform spraying of chemical insecticides for reduction of tick population in green surfaces such as forests, park-forests and parks. The chances of being bitten by a tick can be decreased by taking a number of precautions: avoid tick-infected areas, walk on paths away from vegetation, wear light-coloured clothing so that ticks can be spotted more easily, conduct careful examination of your body and arrange for prompt removal of any ticks and use tick repellents for skin or clothing. Moreover, to reduce the number of ticks in the surrounding nature it is recommended to remove leaves, litter, woodpiles, cut grass and brush from the area<sup>3,4</sup>.

Investigations of ecology of *B. burgdorferi sensu lato*, vectors and reservoirs in our environment have been conducted for more than 20 years. *Ixodes ricinus* (*I. ricinus*) was confirmed as a vector of LB in 1990 in former Yugoslavia<sup>5</sup>. The first isolation of *B. burgdorferi sensu lato* from *Apodemus flavicollis* was performed<sup>6</sup>. All the three pathogens of the complex *B. burgdorferi sensu lato* were isolated in Serbia: *Borrelia sensu stricto*, *Borrelia afzelii* and *Borrelia garinii*. The prevalence of *B. burgdorferi sensu lato* in *I. ricinus* ticks collected in different regions in Serbia between 1990–2005 ranged as follows: Zaječar 26.0 %, Pančevo 19.5%, Zrenjanin 19.8%, Kraljevo 16.1%, Knjaževac 15.4% and Despotovac 33.3%<sup>7</sup>.

Previous studies done in Belgrade estimate that the average infection of ticks with *B. burgdorferi sensu lato* was 21.9%<sup>8</sup>. In the period from 2002 to 2007, the infection rate of *I. ricinus* varied from 17.5 to 21.3% depending on the month and locality<sup>9</sup>. The territory of Belgrade, with numerous green surfaces in various ecological categories (forest, park-forests, parks) and the presence of many host reservoirs (dogs-rambling, game, birds) create an ecological environment fruitful for the appearance of *I. ricinus*, as well as maintaining its population, which thus causes a high occurrence of LB.

In this paper the density of *I. ricinus* ticks and their infection rates with *B. burgdorferi sensu lato* were estimated. We estimated ERI value and how it correlated with tick bites

in people who were present at Belgrade's 19 localities included in the study.

## Methods

The study was carried out in March–October 2009 in Belgrade (Serbia). The research covered two stages of ticks, but only nymphs were used for calculating the entomological risk index (ERI).

### Site selection

The study was carried out at 19 localities in Belgrade classified in three ecological categories: forests (Lipovica, Bojčinska, Avala, Miljakovačka, Makiš), park-forests (Ada Ciganlija, Zvezdara, Banjica, Košutnjak, Jajinci) and parks (Hajd park, Bele vode, Usće, Šumice, Kalemegdan, Topčider, Tašmajdan, Banovo brdo, Pionirski park). All three ecological categories have conditions for maintaining *I. ricinus* tick population.

The parks predominately consisted of annual vegetation and brushes, although there was perennial vegetation, as well. The basic characteristics of the areas were their connection with the surrounding roads and the routine maintenance of the areas throughout the year (pruning trees, mowing lawns, removing leaves, litter accumulations, cut grass, weeds and brush). These areas are also regularly visited by pets and stray dogs. Finally, certain areas in parks are characterized by favorable conditions for the appearance of rodents, squirrels, hedgehogs and birds, too. There are paths, benches and gazebos for persons participating in recreational activities in parks too.

Park-forests are slowly but surely, from year to year changing in favor of parks. A park area is an ideal habitat for ticks, since rodents, lizards and birds live there, where forest areas are characterized by a big number of birds, small and large rodents, reptiles and deer which also host *B. burgdorferi sensu lato*. Park-forests are characterized by the abundance of annual and perennial vegetation.

Forests are covered by perennial deciduous forest vegetation (oak, bitter oak, beech, dogwood, and timber), as well as evergreen vegetation (pine juniper-tree), ivy, mistletoe and annual vegetation. Forests are interspersed with small clearings and roads leading to restaurants. Bojčinska and Makiš forests are located near the Sava and Danube. Throughout the woods there are paths for hikers and vehicles. In the areas of Avala, Bojčinska, and Lipovička forests, there are parts arranged to serve as resting and recreational points (benches, tables, canopies, paths for walking and running).

### Ticks sampling

Ticks were collected monthly in forests (5 localities), park-forests (5 localities) and parks (9 localities), using a flannel cloth of a 1 m<sup>2</sup> surface area (flag hours method) in the duration of 1h. The cloth was checked every 20 min when the attached ticks were removed, counted and placed into humidifi-

ed vials and transported to the laboratory for further investigation. Tick density was expressed by flag/hour (f/h) value – number of ticks collected *per* 1 hour<sup>10,11</sup>.

#### Detection of *B. burgdorferi sensu lato*

Each tick was identified by taxonomic keys and the descriptions of species<sup>12, 13</sup>. The method used to identify the tick infection rate was dark field microscopy in tick midgut tissues with the 400-fold magnification<sup>14</sup>.

#### Entomological risk index (ERI)

ERI was calculated for each locality where the ticks were collected. The ERI value represents the number of nymphs of *I. ricinus* infected with *B. burgdorferi sensu lato* collected *per* minute of flag sampling<sup>15</sup>.

#### Tick bites in humans

Tick bites of nymphs in humans were registered from March to October at each of the 19 localities in Belgrade. The people bitten sought help with the removal of ticks and further prophylactic recommendations from doctors-epidemiologists at the Institute of Epidemiology, Military Medical Academy in Belgrade.

#### Statistical analysis

Analysis of variance (ANOVA) was conducted to compare average tick densities, average tick infection rates and average ERI values between ticks from various ecological categories (fo-

rests, park-forests and parks). Secondary analysis was performed by using Tukey test. In cases of considerable variability of investigated values, Kruskal-Wallis and Mann-Whitney tests were used. The correlations of each locality's ERI value with the number of tick bites in humans was assessed using Pearson's correlation coefficient. Three rates of statistical relevance were considered:  $p < 0.05$ ;  $p < 0.01$ ;  $p < 0.001$ .

#### Results

A total of 3,199 ticks *I. ricinus*, adults and nymphs, were collected from the selected 19 localities in Belgrade. The total infection rate of ticks *I. ricinus* with *B. burgdorferi sensu lato* was 22.0%. Out of all the ticks sampled, 989 (30.9%) were nymphs. The total number of 302 (30.5%) out of all nymphs examined were established infected with *B. burgdorferi sensu lato*. The highest number of nymphs were collected in forests ( $10.65 \pm 4.52$ ), and nymphs in park-forests belonged to the most infected group ( $34.14 \pm 4.82$ ) with *B. burgdorferi sensu lato* (Table 1).

The total number of collected ticks was significantly higher in forests ( $p < 0.05$ ) than parks. The density of nymphs was significantly higher in forests ( $p < 0.05$ ), than park-forests and parks. For ticks originating from various ecological categories no significant difference in infection rates of nymphs was established (Table 2).

ERI value at 19 selected localities was on average 0.49 and varied depending on the ecological category of the locality. The highest average annual ERI value of 1.00 was established for forests, and the lowest for parks 0.19 (Table 3).

The highest ERI values were obtained for Lipovica forest (2.04), park-forest Ada Ciganlija (0.44) and Hajd park (0.38),

**Table 1**  
The total number of collected ticks, only nymphs and percent of nymphs infected at selected localities of Belgrade (f/h)

Ecological category	The total number of collected ticks	Number of collected nymphs	Percent of infected nymphs
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Forests	$31.8 \pm 9.58$	$10.65 \pm 4.52$	$32.12 \pm 6.02$
Park-forests	$21.4 \pm 4.82$	$5.67 \pm 1.24$	$34.14 \pm 4.82$
Parks	$14.9 \pm 9.15$	$4.67 \pm 1.83$	$26.79 \pm 4.92$

**Table 2**  
Comparison of average ticks yearly densities and tick infection rates values among various ecological category (forests, park-forests and parks)

Parameters	ANOVA			Tukey-test
	F	df	p	
Number of collected ticks	6.52	16	0.008	
Forests : park-forests				ns
Forests : parks*				$p < 0.05$
Park-forests : parks				ns
Number of collected nymphs	8.36	16	0.003	
Forests : park-forests*				$p < 0.05$
Forests : parks*				$p < 0.05$
Park-forests : parks				ns
Percent of infected nymphs	3.72	16	0.05	
Forests : park-forests				ns
Forests : parks				ns
Park-forests : parks				ns

\*result statistically significant; ns – non-statistically significant.

**Table 3**  
Average values of entomological risk index (ERI)  
at selected localities of Belgrade

Ecological category	ERI ( $\bar{x} \pm SD$ )
Forests	1.00 $\pm$ 0.69
Park-forests	0.29 $\pm$ 0.11
Parks	0.19 $\pm$ 0.11

and the lowest ERI values were established in Makis forest (0.35), SP Jajinci park-forest (0.20) and in Pionirski park (0.02) (Table 4). A significantly higher ERI value ( $\chi^2 = 7.78, p < 0.01$ ) was found in forests than the parks of Belgrade (Table 5).

In this study, we analyzed ERI values monthly and compared the values established in forests, park-forest and parks. In March and July, we encountered a significantly

higher ERI value in forests, than park-forests ( $p < 0.01$ ) and parks ( $p < 0.01$ ). In the other six months, that is April, May, June, August, September and October, we did not find statistically significant differences in ERI values for all investigated categories (Table 6). May was the month with a statistically significantly higher ERI value for each ecological category, for all the investigated localities (Table 7).

**Table 4**  
Entomological risk index (ERI) values and frequency of  
tick bites in humans on selected localities of Belgrade

Ecological category	ERI	Frequency of tick bites (n)
Forests	1.02	70
Lipovica	2.04	21
Bojcinska	1.03	2
Avala	1.17	26
Miljakovačka	0.53	20
Makiš	0.35	1
Park-forests	0.29	76
Ada Ciganlija	0.44	5
Zvezdara	0.22	16
Banjica	0.22	24
Košutnjak	0.39	21
SP Jajinci	0.20	10
Parks	0.19	83
Hajd park	0.38	27
Bele Vode	0.36	7
Ušće	0.21	15
Šumice	0.20	2
Kalemegdan	0.14	4
Topčider	0.14	21
Tašmajdan	0.15	2
Banovo brdo	0.11	4
Pionirski park	0.02	1

**Table 5**  
Comparison of entomological risk index (ERI) values among ecological categories of Belgrade

Comparison of ecological category	Kruskal-Walis test		
	$\chi^2$	df	<i>p</i>
General analysis	7.94	2	0.02
Forests : Park-forests	3.60	1	0.05 (ns)
Forests : Parks*	7.78	1	0.005 ( $p < 0.01$ )
Park-forests : Parks	2.80	1	0.09 (ns)

\*result statistically significant; ns – non-statistically significant.

**Table 6**  
Entomological risk index (ERI) values analyzed monthly, March and July

Comparison of ecological category	Kruskal-Walis test		
	$\chi^2$	df	<i>p</i>
March (general analysis)	10.3	2	0.006
Forests : park-forests			$p < 0.01$
Forests : parks*			$p < 0.01$
Park-forests : parks			ns
July (general analysis)	9.34	2	0.009
Forests : park-forests*			$p < 0.01$
Forests : parks*			$p < 0.01$
Park-forests : parks			ns

\*result statistically significant; ns – non-statistically significant.

**Table 7**  
**Entomological risk index (ERI) value in May in**  
**ecological categories of Belgrade**

Ecological category	Mann-Whitney test	
	Z	p
Forests*	2.40	< 0.05
Park-forests*	2.61	< 0.01
Parks*	3.57	< 0.01

\*result statistically significant.

Tick bites of nymphs were registered in residents who live or have recreational activities in all 19 localities. All the cases of bites were reported to the Institute of Epidemiology, Military Medical Academy in Belgrade from March to October 2009 (Table 4). In spite of the fact that our results established that the number of tick bites in humans did not correlate with ERI values ( $r = -0.31$ ;  $p = ns$ ), we observed higher ERI values for some localities where the number of registered tick bites was higher. We found the highest frequency of tick bites of nymphs in Banjica (24), Avala (26), Topčider (21), Košutnjak (21) and Hajd park (27). In the ecological category of forests, the lowest number of tick bites (1) was found in Makis, in a number of selected park-forests at Ada Ciganlija (5) and in Pionirski park (1).

### Discussion

In spite of all efforts to establish supervision and control, LB remains the leading arthropod-related disease in the majority of countries around the world<sup>16</sup>. It is caused by spirochete *B. burgdorferi sensu lato* and is associated with the bite of certain *Ixodes* ticks, particularly the *Ixodes scapularis* in the northeastern and north-central United States, *Ixodes pacificus* on the Pacific Coast, *Ixodes persulcatus* in Asia, Europe, Russia, China, Japan, *Ixodes ricinus* in Europe and Euroasia. The secondary vectors of LB are another ticks species: *Haemaphysalis*, *Hyalomma*, *Dermacentor*, *Amblyomma*, *I. hexagonus*, *I. ovatus*, depending on the geographical location<sup>17,18</sup>. All ticks feed on three different host animals during their lives. *I. ricinus* is known to feed on more than 300 different kinds of mammals, birds and reptiles. The genera of rodents *Peromyscus* and *Apodemus* are important reservoirs of *B. burgdorferi sensu lato* in the North America and Europe<sup>19</sup>.

In the USA there are 20,000–24,000 human LB case reports annually<sup>3</sup>. About 60,000 cases are reported each year in Europe<sup>20</sup>. The highest reported frequencies of LB occur in the middle Europe, particularly in Germany 25/100,000, Sweden 69/100,000, Austria 130/100,000 and Slovenia 120/100,000<sup>18,21</sup>. In accordance with the current legislation LB reporting is mandatory in Serbia. As the Institute of Public Health of Serbia "Dr Milan Jovanovic Batut" reports, the number of reported LB cases averaged 784 annually with the incidence of 10.63 /100,000 in 2006–2010<sup>22</sup>. In the period 2011–2012 LB incidence in Serbia averaged 13.49/100,000 per year<sup>23</sup>. In Serbia, as well as in the aforementioned European countries and the USA, LB is diagnosed based on the signs and symptoms of LB, a history of possible exposure to

infected *I. ricinus* ticks and laboratory blood tests which are helpful if used correctly and performed by using validated methods. The two steps of Lyme disease testing are: using a testing procedure called enzyme immunoassay (EIA) or rarely, an indirect immunofluorescence assay (IFA). The second step uses Western blot test (immunoblot test). Results are considered positive only if EIA/IFA and immunoblot are both positive<sup>24,25</sup>.

The risk of LB infection is determined primarily by the density of vector ticks, the prevalence of *B. burgdorferi sensu lato* infection in ticks, seasonal tick activity and the extent of person-tick contact, which is related to the type, frequency, and duration of a person's activities in a tick-infested habitat<sup>4</sup>. Moreover, some ecological factors, as for instance region, climate and landscape, influence the risk of LB infection<sup>26</sup>. The number of ticks varies with geographical position and depends on humidity, daylight and the presence of hosts of infected ticks (wildlife, rodents, birds, stray dogs)<sup>27</sup>. Apart from the abovementioned ecological factors of habitats of infected ticks, in order to assess the risk of *B. burgdorferi sensu lato* infection, some researchers use the value of ERI<sup>26</sup>. It is possible to calculate the ERI value for each separate habitat of infected ticks as an abundance of nymphs (number of nymphs collected per unit of time of sampling) and the local *B. burgdorferi sensu lato* infection rate in nymphs. In the analysis of risk of human LB infection human behaviour and habits formed when on green surfaces should both be taken into consideration<sup>4,28</sup>.

Our results of the density of ticks that ranged from 18.87 to 45.5 f/h in 5 selected forests localities of Belgrade were compared with the results which were obtained by a great number of researchers<sup>11, 29–32</sup>. The comparison shows that the abundance of ticks is the highest in forests, ecotones, flowery vegetation and grassy surfaces. The f/h value at localities depended on the ecological category and varied between 14.9 f/h in parks and 31.8 f/h in the forests. The lowest value 9.75 f/h was found in the park Banovo Brdo and the highest 45.5 in Lipovica forest. The abundance of nymphs was higher in forests, on the average 10.65 f/h, than park-woods 5.67 f/h and parks 4.67 f/h. The density of the total number of ticks and the density of nymphs alone were significantly higher in forests ( $p < 0.05$ ), than park-forests and parks. Our results confirm similar observations in Czech Republic<sup>11</sup> and Belgrade<sup>31</sup> where the f/h values were the lowest (Czech Republic 1986: 2.8–15.1; Belgrade 1993: 4.2–14.2). A smaller number of *I. ricinus* found in parks when compared to other selected localities is due to differences

between ecological categories. Parks have less vegetation, but more concrete paths for recreational activities and walking, regular maintenance, and frequently visiting pets and stray dogs which carry *I. ricinus* on their fur.

During the investigation we found an average tick infection of 22.0% at all selected localities, similarly to our recent results of investigation conducted in Belgrade and the results from Vojvodina and other regions in Serbia<sup>8,9,33</sup>. The infection rates obtained by some researchers (Finland 32.0%, Italy 40.0%, Croatia 45.2%) were higher than in our study<sup>30,34,35</sup> whereas some were lower, in Poland 14% and Denmark 6%<sup>36,37</sup>. The findings about *Borrelia* infection of ticks in Germany (21.8%)<sup>38</sup>. Poland's forests from period 1996–1998 (22.9%)<sup>39</sup> and at workplaces of forestry workers in eastern Poland (22.1%)<sup>40</sup>, are comparable with our data. Researchers from Estonia have found lower values of *B.burgdorferi sensu lato* prevalence 9.7%<sup>41</sup>.

Nymphs are thought to be responsible for the majority of tick bites since they are more numerous than adult ticks and are also more likely to avoid detection when attached<sup>4,18</sup>. Due to that we specially investigated nymph stage and the infection rate of nymphs. The infection rate of *I. ricinus* nymphs may vary from 0% to 66%<sup>42</sup> but mostly varies from 10% to 30%. In our study the infection rate was the highest in park-forests, about 34.1% and the lowest in parks 26.8%, on the average 30.5% in all observed ecological categories, but it did not vary significantly ( $F = 3.72$ ;  $df = 16$ ;  $p = 0.05$ ). Similarly to our results, some authors observed an infection rate nymphs from 13% to 46% and more than 20%<sup>43,44</sup> while others found a lower value of 21.0%, and 4.9–23.1%<sup>45</sup>. In the forests of Germany, researchers observed about five times lower infection rate, 6.9%<sup>46</sup>.

Just like in previous investigations on Belgrade's green surfaces, the ERI value varied at different localities and was 0.02–2.04, but the values in this study were 2–5 times higher<sup>9</sup>. We found a significantly higher ( $p < 0.01$ ) ERI value (1.00) in the selected forests than in the selected parks (0.19) of Belgrade. The values were greater than ERI values obtained by American researchers<sup>15</sup>. Researchers from Vojvodina calculated the highest ERI in the town of Bačka Palanka, 0.158, slightly lower than that obtained from parks in our study<sup>33</sup>. In March and July, we encountered a significantly higher ERI value in the forests than park-forests ( $p < 0.01$ ) and parks ( $p < 0.01$ ). Vegetation in forest is richer than in two others ecological categories and various species of *I. ricinus* hosts live there as well. The ERI value in May was statistically significantly higher for each ecological category, for all investigated localities. That occurred as a result of a greater activity of nymph stage ticks in this period<sup>37,47</sup>.

Similarly to the other observations, which proved a correlation between the ERI value and LB incidence, and a correlation between the ERI value and seropositivity of outdoor workers<sup>9</sup>, we analyzed the correlation between ERI value and the number of tick bites<sup>15,28,48,49</sup>. In spite of a great numbers of registered tick bites, somewhere the highest (in category of parks: Hajd park), the number of tick bites in humans did not correlate with ERI values. In order to explain our results, it is important to take into consideration the other factors that depend on the residents' activity in the selected places (behaviour in the nature and the use of preventive measures for avoiding tick bites or fast detec-

tion of tick bites). Also, it is presupposed that not all people report to a doctor after being bitten by a tick. In rare cases, it is possible for the person not to notice a tick bite, and the tick gets torn off by accident after scratching or releases itself after getting enough blood. Removal of ticks can be carried out by a doctor in any ambulance (which are available at all locations), and again in rare cases, people remove it by themselves and bring it for examination. Still most of the bitten people, report themselves to an epidemiologist in our institution, having in mind that tick testing to *Borrelia* existence is done only in the Military Medical Academy in Belgrade. If *Borrelia* is present in the tested tick, the analysis is positive and it is suggested that the patient see an infectologist who will prescribe antibiotics for LB prevention (therapy lasts 2 weeks). If the person does not bring the tick which bit him or her to get tested and the early signs of infection have already appeared, he or she is immediately sent to an infectologist for further testing, diagnosis and treatment. Also, it should be noted that there are other methods of risk assessment involving tick adults and other environmental habitat parameters, which are applied by some researchers<sup>33,50,51</sup>.

Among the selected five park-forests Košutnjak was the most frequently visited place. A great number of residents take recreational activities there, or go for daily walks because they live near to this park-forest. The ERI value in Košutnjak (0.39) was higher than in Banjica (0.22), but the frequency of tick bites was higher in Banjica. A great number of persons with tick bites who live near Banjica, visit doctors in the Institute of Epidemiology, because the Military Medical Academy is situated in Banjica. On the other hand, a certain number of tick bites from Košutnjak may have remained unregistered, because the persons bitten did not visit a doctor or did not detect ticks on their body. The ERI value at the park-forest Ada Ciganlija was paradoxically high (0.44) compared to just one tick bite, which is explained by the influence of other ecological factors in the environment and factors relating to the activities and behaviour of people<sup>28</sup>. Among the five selected forests the highest ERI (2.04) was calculated for the Lipovica forest and then for Avala (1.17). However, the number of tick bites at Avala (26) was the highest, because that place, as it is arranged and adapted for human leisure activity in a more appropriate manner, is visited by more people than Lipovica. Among the parks, the highest ERI value (0.37) and the number of nymphs (27) were encountered in the Hajd park.

## Conclusion

The study indicates the risk of tick bites, the risk of human *B. burgdorferi sensu lato* exposure and getting infected by LB at all the selected localities in Belgrade. For a more comprehensive Lyme disease risk assessment it is necessary to incorporate other stages of ticks (apart from the nymph stage) into the assessment using appropriate evaluation methods. Also, human behaviour as well as habits of people who visit parks, park-forests and forests of Belgrade should be taken into consideration. With a view to conducting a thorough LB risk assessment, the method of entomological risk index assessment should be combined with other methods taking into consideration all the previously mentioned.

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