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Assessment of Lyme disease risk by using the ecological risk index in the parks of Belgrade

Procena rizika od lajmske bolesti primenom ekološkog indeksa rizika u parkovima Beograda

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Abstract

Background/Aim. Factors determining the risk of Lyme disease (LD) may be followed in terms of the type of habitat. The evaluation of the risk of *Borrelia burgdorferi (B. burgdorferi)* transmission in humans on *Ixodes ricinus* (*I. ricinus)* tick habitats is done by means of the ecological risk index, which determines the tick habitat, abundance and infection rate. The aim of this paper was to determine the value of ecological risk index (potential and actual risk – PR and AR) on green areas in 9 parks in Belgrade and establish the correlation of this index with tick bites in humans. **Methods.** Ticks were collected in parks by means of the flag hour method and examined for the presence of LD cause in dark-field microscopic analysis. Point values were assigned to certain parameters and potential and actual risk index evaluated for each habitat. The data on tick bites from the surveyed habitats were obtained from the Protocol of patients bitten by ticks of the Sector for Preventive Medicine, Institute of Epidemiology, Military Medical Academy in Belgrade. Analysis of variance (ANOVA), Tukey test and Pearson´s coefficient were used in statistical analysis of data. **Results**. In Belgrade´s park habitats a high PR of *B. burgdorferi* transmission was determined, while AR for 4 habitats was categorised as PR, and limited for other 5 habitats. Statistically, in terms of AR values the following habitats were significantly different ($p < 0.05$): Hajd Park and Tašmajdan; Hajd Park and Kalemegdan; Hajd Park and Pionirski Park; Hajd Park and Banovo Brdo; Topčider and Tašmajdan; Topčider and Kalemegdan; Topčider and Pionirski Park; Topčider and Banovo Brdo Park. A statistically significant correlation ($p < 0.05$) between bites of adults and the number of bites of infected adults with the AR value was established. **Conclusion.** In parks of Belgrade, there is a limited AR of *B. burgdorferi* transmission on the average. The AR values vary from limited to the potential, depending on the ecological features of habitat, the number of collected ticks and their infection rate. In view of the correlation of AR with the bites in humans, this index is significant for assessing LD risk.

Key words:
ticks; risk assessment; borelia burgdorferi; disease vectors; serbia.

Apstrakt

Uvod/Cilj. Faktori koji određuju rizik od lajmske bolesti (LB) mogu se pratiti u odnosu na tip staništa. Procena rizika od prenošenja *Borrelia burgdorferi (B. burgdorferi)* kod ljudi na staništima krpelja *Ixodes ricinus* (*I. ricinus)* vrši se preko ekološkog indeksa rizika, koji određuje stanište, brojnost i zaraženost krpelja. Cilj rada bio je da se odredi vrednost ekološkog indeksa rizika [potencijalnog rizika (PR) i aktuelnog rizika (AR)] na zelenim površinama devet parkova Beograda i utvrdi korelacija ovog indeksa sa ubodima krpelja kod ljudi. **Metode.** Krpelji su u parkovima sakupljani metodom *flag* časa i pregledani na prisustvo uzročnika LB u tamnom vlažnom polju mikroskopa. Bodovanjem određenih parametara po odgovarajućim skalama, procenjivani su potencijalni i aktuelni indeks rizika za svako stanište. Podaci o ubodima krpelja sa ispitivanih staništa dobijeni su iz Protokola pacijenata sa ubodom krpelja Instituta za epidemiologiju Sektora za preventivnu medicinu Vojnomedicinske akademije u Beogradu. Pri statističkoj obradi podataka, korišćeni su analiza varijanse (ANOVA), Tukey test i Pearsonov koeficijent. **Rezultati**. Na staništima parkova Beograda ustanovljen je visok PR transmisije *B. burgdorferi*, dok je AR 4 staništa svrstan u kategoriju mogućeg rizika, a na ostalih 5 staništa procenjen je kao ograničen. Po vrednostima AR statistički značajno su se razlikovala ($p < 0,05$) staništa: Hajd park i Tašmajdan; Hajd park i Kalemegdan; Hajd park i Pionirski park; Hajd park i Banovo brdo; Topčider i Tašmajdan; Topčider i Kalemegdan; Topčider i Pionirski park; Topčider i Park Banovo brdo. Utvrđena je statistički značajna povezanost ($p < 0,05$) broja

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uboda odraslih i broja uboda zaraženih odraslih jedinki, sa vrednošću AR. **Zaključak.** U parkovima Beograda, u proseku postoji ograničen AR transmisije *B. burgdorferi*. Vrednosti AR variraju od ograničenog do mogućeg, zavisno od ekoloških karakteristika staništa, broja sakupljenih krpelja i njihove inficiranosti. S obzirom na to da postoji korelacija AR sa ubodima kod ljudi, ovaj indeks je od značaja za procenu rizika od LB.

Ključne reči:
krpelji; rizik, procena; borrelia burgdorferi; bolest, prenosioci; srbija.

Introduction

Lyme disease (LD) has been present in Serbia since 1987, with the constant increase in the number of infected persons [1]. The ticks of *Ixodes* species play a primary role in transmitting the disease, and in our environment specifically *Ixodes ricinus (I. ricinus)* whose bite can infect and make people contract this disease [1–3]. Based on surveys conducted in Belgrade, a number of habitats were marked as natural foci of LD. These habitats carry the risk of bites by infected ticks, especially during the season of their activity, from March until October [4]. This risk increases with the length of exposure to ticks' habitats. People whose regular professional activities are carried out in nature are especially at risk (hunters, forestry workers, workers maintaining green spaces, farmers, veterinarians, soldiers, etc.), as well as those who temporarily visit nature for recreational purposes or walking. In addition to this, the risk of being infected with the spirochete *B. burgdorferi* depends on the abundance of ticks and the rate of infection with the cause of LD in ticks [5, 6]. Past surveys in Belgrade used the entomologic risk index (ERI) [7] to evaluate the risk of infection with *B. burgdorferi*. Ecological risk index was also determined for a number of habitats, but no correlation of the actual risk (AR) index with bites in humans on these habitats was found [8].

The aim of this study was to determine the ecological risk index of transmitting *B. burgdorferi* in green spaces of nine parks in Belgrade, to prove the correlation of this index with bites in humans and based on all this to evaluate the risk of infection with LD.

Methods

The survey encompassed 9 park spaces in Belgrade, with the most frequent occurrence of bites in patients. Ticks were collected monthly by using a 1 m^2 flannel cloth (flag-hour method) for 1 h. It was checked every 20 m and attached ticks were removed, counted and placed in humidified vials and transported to a laboratory for further investigations. Tick density was expressed by the flag hour value (f/h): number of ticks sampled *per* 1 hour [9]. This survey covered adult forms and nymphs [10, 11]. Identification and determination of ticks in laboratory was performed in accordance with taxonomic keys and species descriptions. The presence of *B. burgdorferi* in ticks was detected by dark field microscopy with the magnification rate of × 400 [12].

Ecological index of *B. burgdorferi* transmission was evaluated in the following 9 habitats: Hajd Park, Bele Vode Park, Ušće, Šumice, Kalemegdan, Topčider, Tašmajdan, Banovo Brdo Park and Pionirski Park. The surveyed habitats belonging to the ecological category of parks are characterised by certain ecological features and influenced by a degree of anthropogenic factor, thus differentiating them from other ecological categories (forests, forest parks).

Hajd Park (140 m above sea level, surface area of 7.3 ha) – beside perennial vegetation [plane-tree, oak, hazel, lime-tree, thorny bushes, beech, turkey (bitter) oak, willow, *forsythia*], numerous annual green plants are also present. The park is organised for recreation purposes (trim-trails, exercise equipment). It is surrounded by roads, intercepted with streets linking it with the residential area and the city center. Apart from plants, several types of mouse-like rodents, squirrels and great many birds have been registered in the park. Stray dogs, as well as pets taken out by citizens can be also found there.

Bele Vode Park (139 m above sea level, surface area of 8.0 ha) is divided into two parts by one street. "Ibarska magistrala" main road is located on the upper side of the park. A part of the park is rather derelict, uncared for, covered in shrub vegetation. The remaining part is organised for recreation purposes (playgrounds, children's park with equipment). Perennial woody plants are present, such as: walnut tree, locust tree, chestnut, pine, birch, and *forsythia* of shrub vegetation. Of annual plants there are many sorts of grass from the *Gramineae* family. The presence of mouse-like rodents, squirrels and many birds has been registered in the park.

Ušće Park (89 m above sea level, together with the Park Prijateljstva having 150 ha surface area). Over 90% of the habitat is planned. Ušće is connected with roads to all city parts and facilities on water. There are many recreational trails in Ušće (a bicycle track particularly stands out), great many restaurants, shopping and cultural facilities, and a part of the space is used for public events (concerts). This habitat is comprised of the following vegetation: a poplar tree, pine-tree, weeping willow, *forsythia*, birch-tree, thorny bushes and lime-tree, as well as annual species, mostly grass of the *Gramineae* family. Ušće abounds with animals such as mouse-like rodents, squirrels, as well as numerous birds. Walking the pets is allowed in Ušće, but limited in terms of time. Ušće is an area visited a lot by people.

Šumice Park (130 m above sea level, surface area of 1.0 ha) is mainly under perennial vegetation (plane-tree, common lilac, hazel, oak, *forsythia*, plum). Annual plants from the *Gramineae* family are mainly present, and as for animals: mouse-like rodents, squirrels, pets, stray dogs and many birds. The park is surrounded by roads. Recreational trails are built inside the park, as well.

Banovo Brdo Park (97 m above sea level, surface area of 4.0 ha) is intercepted with numerous trails, with many exercise devices especially tailored for recreational purposes. The park is

comprised of the following vegetation: hazel, pine, chestnut, weeping willow and *forsythia*, and grass of the annual vegetation. The following animals are present: mouse-like rodents, squirrels, stray dogs, pets and many species of birds. The park has good connections with the main road and residential area. There are many hospitality facilities on the outskirts of the park.

Kalemegdan (125.5 m above sea level, surface area of 57 ha), has good connections with all city roads. A fortress is on one side and the Zoo on the other side. Vegetation is comprised of the perennial woody plants: plane-tree, beech, common oak, Turkish hazel, many sorts of conifer trees and bushy vegetation, and various annual plants from the *Gramnineae* family. Beside many fortress visitors, there are many people engaged in sports (basketball, tennis grounds), recreation and guests (pubs, zoo garden). Of animals there are many kinds of birds, squirrels and stray dogs in Kalemegdan.

Topčider (78 m above sea level, surface area of 35 ha) – one half of this space is dedicated to an organised park space and the other half to forests. The perennial vegetation is comprised of: turkey oak, spruce tree, silver fir, oak, plane-tree, lime-tree and cypress, and of bushy vegetation such as: hazel, locust-tree and hawthorn. Annual grass, bushy trees and flowers can be found in the part belonging to the park space. This space is organised with trails, benches and hospitality facilities, thus good many people visit it. Numerous birds, squirrels, hedgehogs, mouse-like rodents live in the park and forest parts.

Tašmajdan (136 m above sea level, surface area of 6 ha) – the vegetation is comprised of perennial woody and bushy plants, as well as annual grass. Besides plane-tree and common oak, horse chestnut is also present. This space is intertwined with walking trails, furnished with benches to relaxation, and the outskirts of park are dedicated for hospitality facilities. It is highly frequented by people during the year.

Pionirski Park (126 m above sea level, surface area of 3.6 ha) – this habitat is comprised of great many common oaks, nettle trees (*celtis australis*), gingko trees, horse chestnuts, annual grass and flowers. The park is organised for relaxation and recreation, with great many trails, benches and street lights. A part of park is cultivated (with flowers), and the other part is under grass. Apart from numerous birds, squirrels and pets, the presence of mouse-like rodents was registered in the park.

In order to evaluate the ecological risk index, a value of potential (PR) and AR of *B. burgdorferi* [13] transmission was determined for each selected habitat of infected *I. ricinus* ticks. The suitability, amount and accessibility of habitat are parameters given point values of 1 to 5 according to the previously prepared chart. The resulting score of those ecological parameters represents the value of potential risk for a certain habitat. PR categories are divided into three groups: I, high risk (11–15 points); II, moderate risk (6–10 points); III, low risk (less than 6 points).

In order to evaluate the AR of *B. burgdorferi* transmission, two more parameters were established: the amount of *I. ricinus* ticks collected in 1 hour, as well as the rate of infection found in them, rated according to the same principle. The total points of all five parameters for one habitat results in the key value of AR of *B. burgdorferi* [13] transmission. The habitats can be divided into five groups according to the number of points: habitats with a definite risk, from 21 to 25 points; habitats with a possible (potential) risk, from 16 to 20 points; habitats with a limited risk, from 11 to 15 points; habitats having no present risk, from 6 to 10 points; habitats having no risk likely, less than 6 points.

The Protocol of patients bitten by ticks from the Institute of Epidemiology, Sector for Preventive Medicine of the Military Medical Academy in Belgrade was used as a source of data on the number of tick bites in people on the surveyed habitats. The protocol keeps records on removed tick bites from patients who were treated in the Institute of Epidemiology, and who were bitten by ticks on the surveyed habitats in Belgrade.

Statistical analysis

Analysis of variance (ANOVA) was conducted to compare average tick densities and average tick infection rates. Secondary analysis was performed by using Tukey test. Correlations between AR values of each locality with the number of tick-bitten humans were assessed by using Pearson′s correlation coefficient. Each p value of < 0.05 was considered statistically significant.

Results

A total of 856 ticks (adult and nymphal forms) were collected from the surveyed Belgrade´s parks during one season of ticks' activity, from March until October. Table 1 shows the average value and standard deviation of the number of collected ticks. There was almost two and a half times greater number of collected adult ticks (11.06 ± 8.64) than nymphs (4.67 ± 1.83).

The average value of flag-hour (f/h) for adult ticks amounted to 11.7 f/h in the surveyed habitats, and 4.68 f/h in case of nymphs. The greatest numbers of adult ticks (30.63 f/h) were collected in Topčider Park, while the number of nymphs was almost seven times smaller (4.50 f/h). In Pionirski Park habitat, flag-hour of nymphs (1.38 f/h) and adult ticks (1.75 f/h) marked the lowest values. The number of adult ticks was approximately two times greater in several park habitats: Banovo Brdo, Kalemegdan, Šumice, Ušće (Table 2).

Table 1

Number of collected ticks in the selected parks

Forms of tics	$\bar{x} \pm SD$
Both forms of ticks	14.9 ± 9.15
Adults	11.06 ± 8.64
Nymphs	4.67 ± 1.83

Table 2

Value of flag-hour (f/h) in parks of Belgrade

Parks in Belgrade	Adults ticks (f/ h)	Nymphs (f/ h)
Hajd park	16.38	6.88
Park Bele vode	11.13	7.38
Ušće	10.25	5.13
Šumice	12.75	5.50
Kalemegdan	6.88	3.75
Topčider	30.63	4.50
Tašmajdan	3.50	4.13
Park Banovo Brdo	6.38	3.38
Pionirski park	1.75	1.38
The average value	11.07	4.68

The ticks collected in the surveyed parks were infected 19.0% on the average. The greatest number of infected ticks, 33.2%, was registered in Hajd Park, and the least one in Šumice (14.1%) and Banovo Brdo (13.8%) (Table 3). Bele Vode and Kalemegdan parks had a similar percentage of infected ticks (21.5% and 22.3%, respectively). Almost the same percentage of infected ticks was registered in Pionirski Park (15.7%) and Tašmajdan (15.9%) habitats, just like Topčider and Ušće that had almost equal representation of infected ticks (16.7% and 17.8%, respectively).

The average value of PR was classified as a high risk category (11.66 ± 1.32) in the surveyed park habitats. The PR value ranged between 10 in Kalemegdan and Banovo Brdo parks to 13 in Bele Vode Park. In Hajd Park, Topčider and Tašmajdan, PR value was rated with 11, while Ušće and Šumice had one point more (Table 3). The average value of AR in all surveyed parks classifies them as limited risk of transmitting LD cause (14.83 ± 2.41). The AR values in habitats were obtained by including the values of the number of collected ticks *per* flag/hour and their infection rate (Table 3). Four habitats (Hajd Park, Bele Vode Park, Šumice, Topčider) were assigned with the possible AR, while other five parks were classified as habitats with limited AR (Ušće, Kalemegdan, Tašmajdan, Banovo Brdo Park, Pionirski Park). The lowest evaluated AR in parks was demonstrated in Kalemegdan (12.13). In Tašmajdan and Banovo Brdo the AR values (12.25) were equal, almost identical to the previous habitat. Slightly higher AR was demonstrated in Pionirski Park (13.5). Ušće Park was rated with 15, while Bele Vode and Šumice were assigned with some more points (16.13). The greatest risk of transmitting the cause of LD was demonstrated in Hajd Park (17.88) and Topčider (18.25).

In terms of the months observed in park habitats, AR value rises from March (Figure 1) and reaches its peak value (19.4) as early as April, then starting to gradually decline without a significant variation in the trend and ultimately reaching the lowest value in October (12.3).

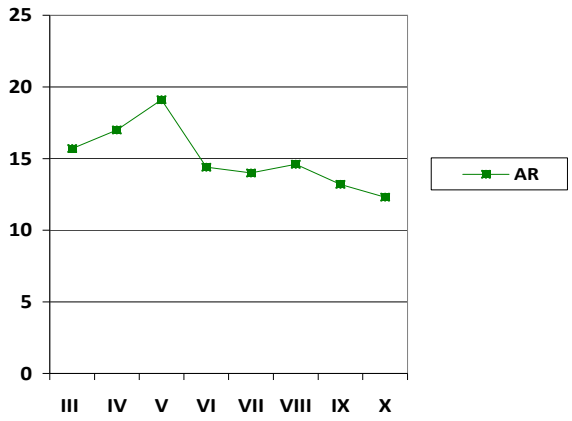

Fig. 1 – The values of actual risk (AR) by months.

Table 3

Number of collected adult ticks (f/ h), percent of infected adult ticks, potential risk and actual risk in the parks of Belgrade

Parks in Belgrade	Number of collected adult ticks (f/ h)	Percent of infected adult ticks (%)	Potential risk	Actual risk
Hajd park	16.38	33.2%	11	17.88
Park Bele vode	11.13	21.5%	13	16.13
Ušće	10.25	17.8%	12	15.00
Šumice	12.75	14.1%	12	16.13
Kalemegdan	6.88	22.3%	10	12.13
Topčider	30.63	16.7%	11	18.25
Tašmajdan	3.50	15.9%	11	12.25
Park Banovo brdo	6.38	13.8%	10	12.25
Pionirski park	1.75	15.7%	12	13.50

f/h – flag-hour.

Of the park habitats, the following habitats were statistically significantly different in terms of AR values: Hajd Park and Tašmajdan ($p < 0.05$); Hajd Park and Kalemegdan ($p < 0.05$); Hajd Park and Pionirski Park ($p < 0.05$); Hajd Park and Banovo Brdo ($p < 0.05$); Topčider and Tašmajdan ($p < 0.05$); Topčider and Kalemegdan ($p < 0.05$); Topčider and Pionirski Park ($p < 0.05$); Topčider and Banovo Brdo Park ($p < 0.05$). This comparison is shown in Table 4. The greatest AR was estimated in Topčider (18.25) and Hajd Park (17.88).

By comparing the AR values between the surveyed park habitats, we came to the conclusion that the values for Hajd Park and Topčider statistically differ ($p < 0.05$) from the values obtained for Tašmajdan (12.25), Kalemegdan (12.13) and Banovo Brdo (12.25), which represent the lowest AR values in the group of the surveyed parks.

On the surveyed habitats, 89 adult tick bites (Table 5) were reported in patients within the period March-October. The greatest number of adult tick bites was reported in Topčider (32), and the lowest number in Pionirski Park (1). In Hajd Park (17) and Ušće (16), the number of tick bites was almost the same, and two times less frequent than in Topčider. In terms of the number of infected adult ticks removed from humans, Hajd Park (7) and Topčider (6) were the leaders, while the ticks sampled in Šumice, Kalemegdan and Pionirski Park were not infected with *B. burgdorferi*.

By applying Pearson's correlation coefficient, a statistically significant correlation between the number of adult tick bites ($p < 0.05$) and the number of infected adult tick bites ($p < 0.05$), with the value of AR (Figure 2) was established.

Table 4

Comparison of actual risk value between localities of parks

Parameters	ANOVA		Tukey-test
	F	*p*	*p*
Actual Risk	6.16	< 0.001	
Hajd Park : Tašmajdan			< 0.05
Hajd Park : Kalemegdan			< 0.05
Hajd Park : Pionirski Park			< 0.05
Hajd Park: Park Banovo brdo			< 0.05
Topcider : Tašmajdan			< 0.05
Topcider : Kalemegdan			< 0.05
Topcider : Pionirski Park			< 0.05
Topcider : Park Banovo brdo			< 0.05

Table 5

Frequency of tick bites in the selected parks of Belgrade

Parks in Belgrade	Number of tick bites (adults ticks)	Number (%) of tick bites (infected adults ticks)
Hajd park	17	7 (41.2)
Park Bele vode	8	1 (12.5)
Ušće	16	3 (18.7)
Šumice	5	0 (0)
Kalemegdan	3	0 (0)
Topčider	32	6 (18.7)
Tašmajdan	2	1 (50.0)
Banovo Brdo	5	2 (40.0)
Pionirski park	1	0 (0)
Total	89	20 (22.5)

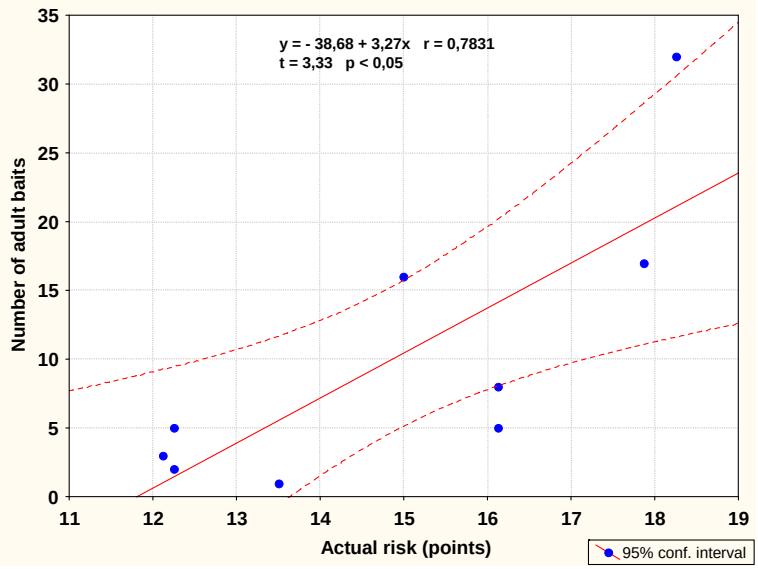

Fig. 2 – Correlation between tick bites of adults ticks and actual risk (AR) in parks.

Discussion

Factors determining the risk of LD may be followed in terms of habitat type, which was the subject of research for many authors [14]. Some scientists apply entomologic risk index [15, 16] to evaluate the risk of infection with *B. burgdorferi* in humans, while others use ecological risk evaluation, *ie* evaluation of PR and AR on Lyme disease vector habitats [14]. The method of evaluating ecological risk considers key ecological parameters (accessibility and the amount of habitat, the composition of habitat in terms of vegetation, suitability of habitat), varying from habitat to habitat, depending on their ecological category, as well as the abundance and the rate of infected adult *I. ricinus* ticks. The assessment of risk by using this method was used in our country in previous surveys made in Belgrade [8] and in the area of Vojvodina [17]. In addition to the assessment of risk index, our survey, unlike past surveys, conducted the comparison of risk index with tick bites in humans in the surveyed habitats.

Abundance of ticks in the specific habitat is one of the factors determining the risk of LD. Abundance of ticks depends on the type of habitat, *ie* ecological category (park, forest-park, forest). Some authors [18] have pointed at the greater abundance of ticks in habitats covered with richer vegetation, with lesser or minimized impact of anthropogenic factors (forest-parks and forests). The category of parks is identified in terms of its ecological features as having mainly annual vegetation, small wild animals, stray dogs, cats, pets taken out for walk by their owners. The human factor has a greater influence on these habitats in terms of a better management of vegetation and the presence of concrete tracks, sport grounds, hospitality facilities, and thus the density of ticks is smaller [19]. Adult ticks are taken into account for the assessment of AR. In the course of our survey, two and a half times greater number of adult forms (11.06 ± 8.64) than nymphal ones (4.67 ± 1.83) was collected. An average value of flag/hour was 11.07 f/h, and it ranged from the minimum value in Pionirski Park of 1.75 f/h to the highest value in Topčider Park of 30.63 f/h. In the survey conducted in Belgrade green areas in 2004, the values of flag/hour were somewhat lower ranging from 1.6–23.4 f/h, with 9.2 f/h. [8] as an average value. During the survey of the risk of infection with LD cause in workers maintaining green areas in Belgrade, the average value of flag/hour in park habitats was even lower, 8.7 f/h on the average, ranging between 8.2 and 9.2 f/h [7]. In 2010 Čekanac et al. [20] obtained higher values of flag/hour, 17.9, in the territory of Belgrade, while in surveyed green areas habitats in Vojvodina, Potkonjak, et al. [17] calculated flag/hour values of 2–80.0 f/h, while flag-hour values in the park next to the railway station (3.0 f/h) and in Kamenički Park (15.0 f/h) were close to our surveys conducted in Tašmajdan (3.50 f/h) and Hajd Park (16.38 f/h).

In our survey, the average rate of infection in *I. ricinus* ticks with *B. burgdorferi* spirochete amounted to 19.0% on the average, ranging in habitats from 13.8% in Banovo Brdo Park, to the highest of 33.2% in Hajd Park. In surveys made by Krstić and Stajković [8], the infection rate in Hajd Park was almost twice lower (19.8%), and four times greater in Tašmajdan (4.0%) compared to our results. These data point to the fact that the tick infection rate on a habitat is not a constant value, but prone to changes depending on the season, presence and diversity of reservoirs and other ecological factors [20–23]. During the survey conducted in 2007 [7], ticks in Belgrade´s parks were infected 13.65% on the average, while Potkonjak, et al. [17] published the infection prevalence in ticks from 0–33.1% in the territory of Vojvodina. Stajković, et al. [4] found the infection prevalence in *I. ricinus* of 27.0–31.7% in Belgrade´s green areas. Estonian authors [24] detected lower values of *B. burgdorferi* prevalence (9.7 %), while Romanian researchers [25] registered the infection in ticks on 183 sites ranging from 0.75% to 18.8%.

Determination of ecological risk encompasses the assessment of PR and AR in one habitat. By rating the appropriate parameters that determine PR (habitat suitability, amount and accessibility) we obtained PR values for each surveyed park. In view of the fact that PR values of 9 Belgrade´s parks were not mutually significantly varied, based on their average value, these habitats were categorised as having high risk (11.66 ± 1.32). PR ranged from the lowest value in Kalemegdan and Banovo Brdo parks (10) to the highest in Bele Vode Park (13). Similarly, in the previous survey in 2004 conducted on the majority of Belgrade´s habitats the PR was classified as high, as 13 localities had high PR, and only 3 moderate. In line with our results, Potkonjak, et al. [17] registered also high PR of LD cause transmission in Vojvodina in 8 of 12 surveyed habitats in 2011.

Aimed at assessing the AR of *B. burgdorferi* transmission, beside ecological parameters (suitability, amount, accessibility of habitat) we rated two more parameters: abundance of *I. ricinus* collected during 1 h and their infection rate with *B. burgdorferi* [14]. A total of points for all the mentioned parameters resulted in the AR value of the specific habitat. Four park habitats in Belgrade (Hajd Park, Bele Vode Park, Šumice, Topčider) were classified as potential AR, while other five (Ušće, Kalemegdan, Tašmajdan, Banovo Brdo Park, Pionirski Park) were evaluated as habitats with a limited AR of LD cause transmission. Since ecological parameters are specific for each habitat, and the abundance and tick infection rate vary depending on the season, climate factors, the presence, type and the number of *B. burgdorferi* reservoirs, our AR values are different for each habitat, and the months in the season of tick activity. The average AR value in Belgrade´s park habitats starts to rise at the beginning of season – March, and reaches its maximum value in April, then gradually starts to decline with some variation until October. The parks with the highest AR values are: Hajd Park with 17.88 and Topčider with 18.25, they are in terms of statistics significantly different ($p < 0.05$) than the parks of Kalemegdan (12.13), Tašmajdan (12.25), Banovo Brdo (12.25) and Pionirski Park (13.50), where the lowest number of *I. ricinus* ticks was collected. Similarly, the authors from Vojvodina [17] determined a limited AR for the majority of surveyed habitats (six of twelve), while evaluated a definite AR of *B. burgdorferi* transmission in three habitats, unlike our survey that did not detect habitats with the definite

AR. In past were surveys conducted in the territory of Belgrade (2004) [8], AR ranged from the limited to the definite in surveyed parks and varied depending on the month of survey.

The number of tick bites in patients from the surveyed habitats was used to evaluate the importance of AR determination in habitats. During the season of tick activity, 89 bites by adult ticks were registered in patients, with the *B. burdorferi* prevalence of 22.5%. Almost the same infection rate in removed ticks in Belgrade territory (2010) was detected by Mladenović et al. [26] (21.25%). The greatest number (32) of bites was registered in Topčider, where the AR value was the highest. The removed tick infection rate was the highest in ticks from Hajd Park (7) and Topčider (6), which, in terms of AR value, were statistically significantly different from other parks. In order to evaluate the LD risk, some authors have determined a correlation between the density or prevalence of ticks with seropositivity or the appearance of LB symptoms [27–30], while some determine the correlation of entomologic risk with the Lyme disease incidence [31]. We compared the number of adult tick bites and the number of infected adult tick bites with the AR values on the same habitats of bite occurrence. The correlation between tick bites and AR of *B. burgdorferi* transmission ($p < 0.05$) suggests that the method of assessing ecological risk is useful in LD risk assessment.

Conclusion

In Belgrade´s parks there is a limited AR of *B. burgdorferi* transmission on the average. AR values vary in parks from limited to possible, depending on the ecological features of habitats, the number of collected ticks and their infection rate. In view of the correlation of AR with bites in humans, this index is a significant one for the assessment of LD risk.

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