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Full Research Article

BORELLIA BURGDORFERI INFECTION IN REMOVED TICKS AND ANTI-*BORRELIA* ANTIBODIES IN INFESTED PATIENTS ADMITTED TO THE PASTEUR INSTITUTE, NOVI SAD

SIMIN Verica[1*], LALOŠEVIĆ Dušan[1], MIJATOVIĆ Dragana[2, 3], TOMANOVIĆ Snežana[4] MILJEVIĆ Milan [5], ČABRILO Borislav[6], BOGDAN Ivana[1], BANOVIĆ Pavle[2,7]

[1]Pasteur Institute Novi Sad, Department for Microbiological and other Diagnostics, Hajduk Veljkova 1, Novi Sad, Serbia;
[2]Pasteur Institute Novi Sad, Department of Prevention of Rabies and other Infectious Diseases, Clinic for Lyme Borreliosis and other Tick-Borne Diseases, Hajduk Veljkova 1, Novi Sad, Serbia;
[3]University of Pristina – Kosovska Mitrovica, Faculty of Medical Sciences, Anri Dinana bb, Kosovska Mitrovica, Serbia;
[4]University of Belgrade, Institute for Medical Research, Dr Subotića Starijeg 4, Belgrade, Serbia;
[5]University of Belgrade, Institute for Biological Research Siniša Stanković, National Institute of the Republic of Serbia, Department of Genetic Research, Bulevar Despota Stefana 142, 11060, Belgrade, Serbia;
[6]University of Novi Sad, Faculty of Science, Department of Biology and Ecology, Trg Dositeja Obradovića 3, Novi Sad, Serbia;
[7]University of Novi Sad, Medical Faculty Novi Sad, Department of Microbiology with Parasitology and Immunology, Hajduk Veljkova 1-3, Novi Sad, Serbia

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Abstract

The primary objectives of this study were (1) to determine the presence of *Borrelia burgdorferi* infection in ticks removed from patients for the purpose of singling out sites with increased risk of Lyme borreliosis, and (2) to determine the presence of IgM and/or IgG antibodies against *B. burgdorferi* sensu lato (s. l.) complex in sera of patients who had ticks removed. From 108 ticks removed from patients, all were examined

*Corresponding author – e-mail: luketic.s@mts.rs

zoologically and a sub-sample of 91 ticks was tested using PCR analysis to determine the presence of DNA indicating *B. burgdorferi* infection. To detect anti-*Borrelia* IgM and/or IgG antibodies in 61 patients bitten by ticks, we used line recombinant immunoblot test. The most common tick identified was *Ixodes ricinus*. *B. burgdorferi* s. l. was present in 37 of 91 tested ticks (40.7%). Seroconversion against *B. burgdorferi* s. l. antigen was detected in 12 of 61 patients (19.7%). Most of the infected ticks were from the province of Vojvodina (11 municipalities), with the city of Novi Sad proving to be the site with the highest number of infected ticks, 6 in total.

Key words: *B. burgdorferi* sensu lato, *Ixodes*, Lyme borreliosis, serosurvey, ticks, Vojvodina

INTRODUCTION

Lyme borreliosis is a multisystemic tick-borne disease caused by bacteria from the *Borrelia burgdorferi* sensu lato complex (*B. burgdorferi* s. l.). In Europe, the dominant role in transmitting and maintaining the causative agent of Lyme borreliosis belongs to the hard ticks from the genus *Ixodes*, primarily *Ixodes ricinus* (Jensen et al., 2000). The life cycle of this tick comprises three stages: larva, nymph and adult. According to available data in Serbia, a third of the *I. ricinus* population in the country is infected with *B. burgdorferi* (Potkonjak, 2018).

The *B. burgdorferi* s. l. complex contains around 18 species and two genomospecies. The total number of species is subject to change, as new isolates are continually being described on a genetic and antigenic basis. The species with the highest pathogenic potential for humans are *B. afzelli*, *B. burgdorferi* sensu stricto (s. s.), *B. garinii* and *B. spielmanni*. The species *B. bissetti*, *B. valaisiana* and *B. lusitaniae* are considered only mildly pathogenic for humans (Rudenko et al., 2011).

In Europe, the primary reservoirs of the *B. burgdorferi* s. l. complex are various species of mammals (hedgehogs, mice, voles, squirrels, dormice and rabbits), birds (blackbirds, pheasants, auks and snipes) and certain reptiles. Once infected via tick bite, small rodents become a permanent source of infection for other ticks, particularly larvae and nymphs (Mannelli et al., 2012).

Lyme borreliosis can be confirmed through direct and indirect methods. Directly, the presence of *Borrelia* can be demonstrated by cultivation of the spirochetes on specific growth media, using antigen detection via direct immunofluorescence on the intestinal contents of ticks (Ružić-Sabljić and Cerar, 2017). Polymerase chain reaction (PCR) can be used to detect the spirochete DNA in the ticks themselves, as well as in clinical material. Indirect methods for confirming *Borrelia* infection include PCR and serological tests for *Borrelia*-specific antibodies in the serum, cerebrospinal or synovial fluid. IgM and IgG antibodies can be detected via indirect immunofluorescent test (IFA), enzymic immunological tests (ELISA) or immunoblot tests (WB).

The primary objectives of this study were to: (1) find potential geographical localities with increased risk for Lyme borreliosis by determining the presence of *B. burgdorferi* infection in ticks removed from patients, and; (2) determine the presence of IgM and/

or IgG antibodies against *B. burgdorferi* s. l. complex in sera of patients who had ticks removed.

MATERIALS AND METHODS

Tick collection

A total of 108 individual ticks were removed and collected from the skin of patients who were infested with ticks while engaged in outdoor activities from March to October 2018. Ticks were individually stored in sterile plastic tubes and labeled until species determination. Information on date and location of attachment were collected.

Sera collection

In order to detect and identify *B. burgdorferi* s. l. complex antibodies via the line recombinant immunoblot assay (Mikrogen Diagnostik, Germany), a total of 61 blood samples were collected over the course of 2018 from patients from which a tick was removed and analyzed initially. The blood was sampled at least one month after the tick bite.

Species identification and developmental stage determination of ticks

Species and development stage identification of ticks was conducted in the Parasitology Laboratory of the Pasteur Institute immediately after tick collection. The ticks were examined under a stereomicroscope (ST-30-2LR Bino Stereo, magnification 20-40×). Identification was based on morphological characteristics, according to the key by Estrada Pena et al. (2004). After identification, ticks were individually placed in Eppendorf tubes with 70% ethanol until further analysis.

DNA extraction from tick samples

Ticks for molecular analysis were randomly chosen from the pool of 108 ticks to make sample size n=91. DNA was extracted from individual ticks via alkaline lysis with 50 mM NaOH. According to the protocol, prior to extracting the tick from the Eppendorf tube, the 70% ethanol was removed with a pipette and the tick was rinsed twice in phosphate-buffered saline (PBS), pH 7.4. The PBS was then removed and 50 mM of NaOH was added in a volume equal to the volume of the tick. The tick was macerated, vortexed and suspended via centrifuge at high rotation, and then heated in the NaOH in a thermoblock at 95°C. The DNA in the heat-treated sample was neutralized with 1M Tris-HCl (pH 7.0), vortexed and centrifuged. The extracted DNA was transferred to a new Eppendorf tube and its concentration was measured via fluorimeter, after which it was stored at -70°C until further use.

Molecular detection of *Borrelia burgdorferi* sensu lato from tick samples

The presence of *B. burgdorferi* s. l. DNA was confirmed for individual tick DNA samples. For PCR amplification of the 16S rRNA gene, a commercial kit (Norgen Biotek, Canada) for *B. burgdorferi* s. l. detection was used. Amplification of the selected 277 bp sequence was carried out in a Techne TC-PLUS Thermal Cycler in 40 cycles consisting of the following: denaturation at 94°C (15 seconds), hybridization at 60°C (30 seconds) and elongation at 72°C (45 seconds).

Line recombinant immunoblot assay (LRIT)

To determine the presence of *B. burgdorferi* s. l. complex IgM and/or IgG antibodies in human sera, commercial line recombinant immunoblot kits (recomLine *Borrelia*, Mikrogen Diagnostik, Germany) were used. These highly specific and sensitive tests use recombinant proteins as antigens in order to detect five species of the *B. burgdorferi* s. l. complex: p100 – *B. afzelii*, VlsE – several genomospecies, p41 – *B. burgdorferi* s. s., p39 – *B. afzelii*, OspA and OspC – *B. burgdorferi* s. s., *B. afzelii, B. garinii, B. spielmanii*, p18 – *B. burgdorferi* s. s., *B. afzelii, B. garinii, B. spielmanii, B. bavariensis*.

Interpretation of LRIT

The LRIT result was obtained by allotting points to individual bands, as per the manufacturer's recommendations. The number of points remains the same regardless of reaction intensity (noted as +, ++ or +++). The number of points for OspC and p18 bands is calculated only once, even when more than one or all OspC or p18 bands react. Finally, based on the total points, a positive (>7 points), borderline (6 points) or negative (≤5 points) result is given. The evaluation of total points for specific antigens in the recomLine *Borrelia* test for reliable and fast result estimation is shown in Table 1.

Table 1. Number of points for specific *B. burgdorferi* antigens in the recomLine *Borrelia* test (Mikrogen Diagnostik, Germany)

Antigen	points (IgM)	points (IgG)
p100	5	5
VlsE	5	5
p58	4	4
p41	1	1
p39	4	5
OspA	5	5
OspC	8	5
p18	5	5

Statistical analysis

To ascertain if there were statistically significant differences in representation of specific tick developmental stages, the Chi-square test was performed in Microsoft Excel 2013 v.15.0 included in Office 2013 package. The threshold for statistical significance in the differences observed was 0.05.

RESULTS

Tick species and developmental stage identification

All the examined ticks (n=108) belonged to the family Ixodidae, with the species *I. ricinus* accounting for 102 ticks. The remaining ticks belonged to three other species: *Rhipicephalus sanguineus* (3 individuals), *Dermacentor marginatus* (2) and *Haemaphysalis punctata* (1). Of the *I. ricinus* ticks, 51 were nymphs, 36 were adult females and 15 were larvae. Two adult females and one nymph were found in the *R. sanguineus* sample. Both *D. marginatus* individuals were nymphs, and the single *H. punctata* was an adult female.

The highest number of ticks originated from the Novi Sad (54) and Irig (11) municipalities. A smaller number of ticks were collected from Beočin (7) and Sremski Karlovci (5), and the remaining 31 ticks originated from other localities in Serbia and four other countries (Croatia, Austria, Germany and Bosnia and Herzegovina) (Figure 1). The ticks from the Novi Sad municipality consisted of 7 larvae, 27 nymphs and 20 adult females, with the Chi-square test demonstrating a statistically significant difference in the representation of specific developmental stages (p=0.003) (Figure 2).

Tick species	Probable locality of tick attack (municipality)	Number of samples
Ixodes ricinus	Novi Sad	54
Ixodes ricinus	Sremski Karlovci	5
Ixodes ricinus	Sremska Mitrovica	1
Ixodes ricinus	Ruma	1
Ixodes ricinus	Irig	11
Ixodes ricinus	Inđija	2
Ixodes ricinus	Beočin	7
Ixodes ricinus	Fruška gora Mountain (municipality N/A)	3
Ixodes ricinus	Bački Petrovac	1
Ixodes ricinus	Bečej	1
Ixodes ricinus	Vrbas	2
Ixodes ricinus	Apatin	1
Ixodes ricinus	Subotica	1
Ixodes ricinus	Kovin	1
Ixodes ricinus	Čačak	1
Ixodes ricinus	Gornji Milanovac	1
Ixodes ricinus	Ljubovija	1
Ixodes ricinus	Sokobanja	1
Ixodes ricinus	Bajina Bašta	1
Ixodes ricinus	Austria (municipality N/A)	1
Ixodes ricinus	Germany (municipality N/A)	10
Ixodes ricinus	Ilok (Republic of Croatia)	2
Ixodes ricinus	Banja Luka (Bosnia and Herzegovina)	2
Haemaphysalis punctata	Sabac	1
Dermacentor marginatus	Temerin	2
Rhipicephalus sanguineus	Novi Sad	2
	Bečej	1

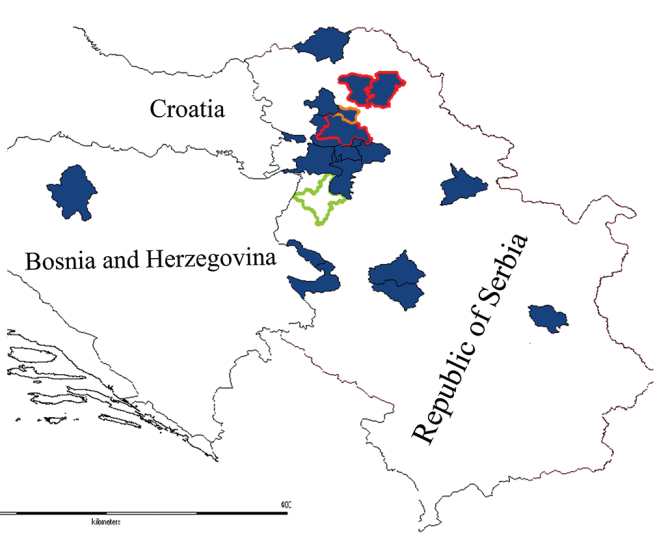


Figure 1. Sites where Pasteur Institute Novi Sad patients were exposed to tick bites over the course of 2018.

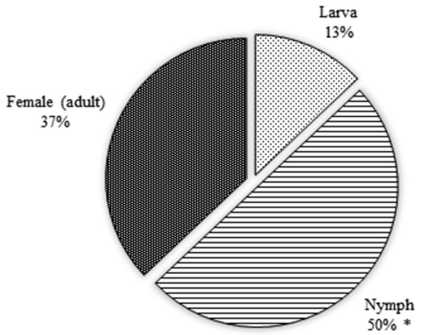


Figure 2. Developmental stage distribution in *I. ricinus* ticks removed from patients where suspected contact was in Novi Sad. An asterisk denotes significant statistical difference (p=0.003).

The presence of *Borrelia burgdorferi* s. l. in ticks

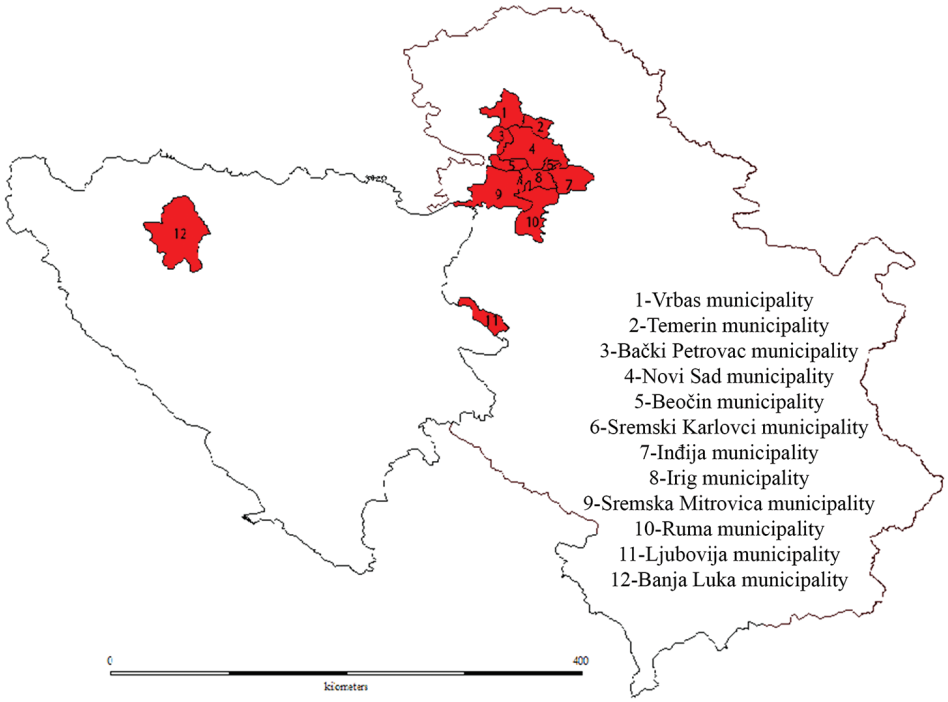
A total of 91 ticks were tested for the presence of *B. burgdorferi* s. l. DNA using PCR: 86 *I. ricinus*, 2 each of *D. marginatus* and *R. sanguineus* and 1 *H. punctata. Borrelia burgdorferi* s. l. DNA was detected in 37 ticks (40.7%) (Table 2, Figure 3). With the exception of a single *D. marginatus*, DNA examination revealed all of the infected ticks were *I. ricinus*. Ticks infected with *Borrelia* originated from 12 municipalities in Serbia and one municipality in Bosnia and Herzegovina, and they belonged to different developmental stages. Of the 37 infected ticks, the majority were nymphs (20, or 54.1%). Twelve adult females (32.4%) and five larvae (13.5%) were also infected with *Borrelia.*

Table 2. *Borrelia burgdorferi* s. l. DNA detection in ticks via PCR analysis

Tick species	Number of samples per species	PCR positive (% of total tick sample)	PCR negative (% of total tick sample)
Ixodes ricinus	86	36 (39.6%)	50 (54.9%)
Dermacentor marginatus	2	1 (1.1%)	1 (1.1%)
Rhipicephalus sanguineus	2	0	2 (2.2%)
Haemaphysalis punctata	1	0	1 (1.1%)
Total	91	37 (40.7%)	54 (59.3%)

The majority of *Borrelia*-positive ticks from Serbia originated from the northern part of the country, the autonomous province of Vojvodina (11 municipalities), and one municipality in western Serbia (Ljubovija) (Figure 3). In Vojvodina, *Borrelia* infections were particularly common in ticks from different sites in the Srem (Syrmia) region, especially around Mt. Fruška Gora (Bukovac, Popovica, Stražilovo, Banstol, Rakovac-Beočin, Ledinci, Irig-Vrdnik-Iriški venac, Ruma). In the Bačka region, *Borrelia*-positive

ticks were found in four municipalities (Novi Sad (19), Temerin (1), Vrbas (2) and Bački Petrovac (1)), with the urban center of Novi Sad containing the highest number of infected ticks, a total of 6.



Tick species	Localities (municipalities)	The number of ticks tested	The number of ticks infected with B. burgdorferi
Ixodes ricinus	Novi Sad	**42**	**19**
Ixodes ricinus	Sremski Karlovci	**5**	**1**
Ixodes ricinus	Sremska Mitrovica	**1**	**1**
Ixodes ricinus	Ruma	**1**	**1**
Ixodes ricinus	Irig	**10**	**5**
Ixodes ricinus	Inđija	**2**	**1**
Ixodes ricinus	Beočin	**6**	**2**
Ixodes ricinus	Fruška gora Mountain	**2**	**1**
Ixodes ricinus	Bački Petrovac	**1**	**1**
Ixodes ricinus	Vrbas	**2**	**2**
Ixodes ricinus	Ljubovija	**1**	**1**
Ixodes ricinus	Banja Luka	**2**	**1**
Dermacentor marginatus	Temerin	**2**	**1**

Figure 3. Geographical locations of *Borrelia*-infected ticks and the results of PCR analysis of ticks for the presence of *Borrelia burgdorferi* s. l. DNA

Testing for IgM and/or IgG antibodies in patients infested with ticks

The presence of IgM or IgG antibodies against *B. burgdorferi* s. l. was recorded in the serum of 12 of the 61 patients tested (19.7%) (Figure 4).

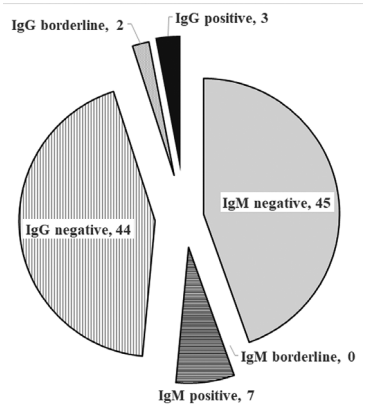


Figure 4. Seroreactivity of patients' sera against *B. burgdorferi* s. l. antigen in LIRT

DISCUSSION

This paper outlines the results of *B. burgdorferi* s. l. presence in ticks and the prevalence of seropositive individuals, patients who were exposed to tick bites. According to the results from the study period (March-October 2018), patients treated at the Clinic for Lyme Borreliosis and other Tick-Borne Diseases, Pasteur Institute Novi Sad, were exposed to bites of four tick species: *I. ricinus*, *R. sanguineus*, *D. marginatus* and *H. punctata*. A similar assemblage of tick species was registered on several sites in the Vojvodina area in prior research (Lalošević and Ivetić, 2011; Savić et al., 2010).

With regards to the developmental stage of the ticks, most of the patients were exposed to nymph bites (54). More nymphs than other developmental stages were collected from patients infected in the Novi Sad area. An explanation can be found in the overlap between the maximum activity of the nymphs, which are the most aggressive stage in the tick life cycle, and extended periods of human outdoor activity in springtime. Due to their small size, larvae and nymphs are difficult to spot. They feed for 2-4 and 4-6 days respectively, which leads to increased risk of *Borrelia* transmission, and then detach from the skin of their host (Banović et al., 2019b). The three Metastriata species are far less frequently found in Serbia than *I. ricinus*. The assemblage of ticks noted in the current study is typical of forest ecosystems of Mt. Fruška Gora, from where most of the studied ticks originated. These habitats possess diverse plant and animal communities that are under low anthropogenic pressure, providing favorable conditions for the maintenance of tick life cycles. However, some of the patients observed/treated for tick bites were from urban areas with high anthropogenic pressure. In habitats such as these, ticks can survive in favorable areas with adequate microclimatic conditions, such as parks, open grassy areas and other plant surfaces that are not regularly treated against ticks.

The prevalence of *Borrelia* infection in ticks, calculated as a percentage of infected ticks in any given period, is the most important parameter for evaluation of risk for

acquiring Lyme borreliosis. In the current study, *B. burgdorferi* s. l. was present in the 40.7% (37/91) of the total tick sample. When observed per tick species, in *I. ricinus* 36 of 86 samples were positive (41.9%); for *D. marginatus* – 1 positive of 2 samples; for *R. sanguineus* – 0 positive of 2 samples; for *H. punctata* – 0 positive of 1 sample. The difference between *Borrelia* infection prevalence in *I. ricinus* and *D. marginatus* is the consequence of their behavior towards hosts. *I. ricinus* is an exophilic species of open grassland and forest ecosystems which are widely distributed in Serbia, while *D. marginatus* is an endophilic species. This tick is far less abundant in Serbia, and it typically hides in bushes, most frequently under pine or oak trees, or in dens and nests of mammals and birds that serve as its hosts (Estrada-Pena et al., 2004).

The ticks originated from several sites in Serbia, as well as four other countries (Germany, Austria, Croatia, Bosnia and Herzegovina). If we focus on Serbia, 41.9% of ticks were PCR positive for the bacteria, a higher prevalence compared to earlier reported values: 22.12% (Savić et al., 2010) and 21.13% (Potkonjak et al., 2014). In the Belgrade area and its adjacent municipalities, reported values of *Borrelia* prevalence in ticks range from 21.9% to 31.7% (Čekanac et al., 1993; Milutinovic et al., 2006). On the other hand, a different study (Tomanović et al., 2010) found that over 40% of *I. ricinus* ticks were infected with these bacteria, similar to the results of the current study. According to prior studies (Milutinovic et al., 2008; Radulović et al., 2010; Tomanović et al., 2010), the following *Borrelia* species are found in Serbian ticks: *B. burgdorferi* s. s, *B. afzelii*, *B. garinii*, *B. valaisiana*, and, most frequently, *B. lusitaniae*. A clinical case of a patient with *B. spielmanii* infection was also previously reported (Banović et al., 2019a).

When interpreting the results of the current study, physical (climatic) and biotic factors of the study area must be considered. These include amount of seasonal rainfall, temperature and light regime and especially relative humidity, as well as use of chemical treatments and other anthropogenic influences, including maintenance of greenery in urban areas. All these factors can interact to affect tick abundance and pathogen prevalence. According to data from the Republic Hydrometeorological Institute of Serbia (Republic Hydrometeorological Service of Serbia, 2019), 2018 was the warmest year since meteorological records began, with the highest minimal spring and summer temperatures and moderate rain in Serbia as a whole. Higher quantities of rainfall were noted in west, southeast and central parts of Serbia. May and June experienced higher than average rainfall, and the rainiest month was July, which is not typical of rainfall patterns in Serbia, an area of temperate continental climate (Republic Hydrometeorological Service of Serbia, 2019). Such climatic conditions exerted a positive influence on the abundance and activity of nymphs and adult ticks during spring and summer of 2018. Consequently, more people than usual were exposed to tick bites in this period, as is consistent with the results of our research.

Numerous studies confirmed the presence of *B. burgdorferi* in other European countries and determined factors that influence the varying prevalence of this spirochete in ticks. These include the availability of adequate tick reservoirs, characteristics of the dynamics of the enzootic cycles, habitat type, elevation, geographic distribution of

principal vectors, and most importantly, geographic distribution of *Borrelia* species. According to Stünzner et al. (2006), tick infection prevalence is reduced with an increase in elevation. In the current study, most infected ticks originated from Mt. Fruška Gora and the Novi Sad municipal area, which are less than 539 m above sea level (this being the elevation of the highest peak on Fruška Gora, Crveni Čot). Golubić and Zember (2001) noted *Borrelia* prevalence (45%) similar to ours (41.9%) in Croatia, where geographic and climatic characteristics are similar to the Srem region in Vojvodina.

Nymphs were the developmental stage most commonly infected with *Borrelia* in the current study (54.1% of infected ticks). Nymphs are considered responsible for infecting humans with the causative agent of Lyme borreliosis in the majority of cases (Steere et al., 2016), as demonstrated in the current study. The higher risk of contracting the disease from nymphs than from other stages results from a combination of two factors: a higher *Borrelia* prevalence in nymphs than in larvae, and the nymphs spending more time feeding on the host (along with smaller size and lesser visibility) than the adults. However, in a similar study of ticks collected from vegetation in the Czech Republic (Hubálek et al., 2003), the authors noted a higher prevalence of *Borrelia* in male *I. ricinus* (26.1%) than in females (24.9%) or nymphs (16.8%), which contrasts with the results of the current study. A second group of Czech authors arrived at a similar conclusion after seven years of monitoring *Borrelia* prevalence in *I. ricinus*: prevalence was highest in males (10.3%), with lower prevalences in females (6.9%), nymphs (5.6%) and larvae (1.6%) (Žákovská et al., 2008).

Factors influencing infection risk in humans include the presence and abundance of ticks in different types of habitat, *Borrelia* prevalence in ticks, and extended periods of possible exposure to ticks. The line recombinant immunoblot assay, performed one month after exposure to tick bites, demonstrated that sera sampled during the early infection stage predominantly contained IgM antibodies, as opposed to sera sampled in the late infection stage where IgG is dominant. The early, or IgM stage of the disease, is characterized by reactions with OspC; in the late IgG stage, strong reactions occur with recombinant proteins (p100, VlsE, p58, p39 and p18). VlsE is an early marker of IgG response, but it is also found in late stages of Lyme borreliosis, along with p100 and/or p18 (Bušová et al., 2018). With this in mind, IgM-positive patients were likely in the early infection stage, as a strong response to OspC was noted in all of them. A positive IgG result for three patients could signify active infections, if the clinical presentation pointed to Lyme borreliosis. Since these patients were asymptomatic, the results indicated either latent *B. burgdorferi* infections, or prior exposure to *B. burgdorferi* s. l. antigens. To demonstrate disease progression or post-therapeutic recovery, the ELISA test for peptide C6 can be performed. On the other hand, a negative IgG or IgM immunoblot result does not exclude the possibility of *B. burgdorferi* infection. Occasionally, antibodies cannot be detected in the early stage of the disease due to their low quantity. If the clinical presentation is indicative of Lyme borreliosis, and the

results of serological tests are negative or dubious, it is advised to perform the tests again in three weeks, while initiating therapy beforehand.

Huegli et al. (2011) reported that 36.8% of ticks removed from patients in an endemic region for Lyme borreliosis – Neuchatel, Switzerland – were infected with *Borrelia*, and that 4.46% of infected patients reached seroconversion (in IgM or IgG class). A study in Sweden and parts of Finland reported that 28% of ticks removed from patients were infected with *Borrelia*, and that 3.5% of patients achieved seroconversion (Wilhelmsson et al., 2016). Here, we report a higher prevalence of tick infection with *Borrelia* (40.6%) as well as more frequent seroconversion in our patients (19.7%) compared to reports from Sweden, Åland Islands and Neuchatel. Possible reasons for higher seroconversion in the current study should be further investigated and may lie in the patient demographic characteristics and in the duration of tick feeding on humans, since in Neuchatel, ticks were removed relatively quickly, within 36 h of attachment.

CONCLUSION

One hundred and eight specimens of ticks removed from the skin of patients at the Pasteur Institute in Novi Sad were identified as belonging to four species: *I. ricinus* (n=102), *R. sanguineus* (n=3), *D. marginatus* (n=2) and *H. punctata* (n=1).

The nymphs were the most common developmental stage found on patients in the Novi Sad area.

Most of the infected ticks were from the AP Vojvodina (11 municipalities) and one municipality in Western Serbia (Ljubovija). In Vojvodina, infected ticks originated from different localities of Srem, mainly Fruška Gora (Bukovac, Popovica, Stražilovo, Banstol, Rakovac-Beočin, Ledinci, Irig-Vrdnik-Iriški venac, Ruma), belonging to the municipalities of Novi Sad, Beočin, Sremski Karlovci, Ruma, Sremska Mitrovica and Inđija. In Bačka region, PCR-positive ticks were registered from four municipalities (Novi Sad, Temerin, Vrbas and Bački Petrovac), with the city of Novi Sad proving to be the site with the highest number of infected ticks, six in total.

In the total sample of infected ticks, the largest number belonged to the nymph stage (20 ticks, or 54.1%).

The presence of IgM or IgG antibodies against *B. burgdorferi* s. l. was recorded in the sera of 12 of the 61 patients tested (19.7%).

Data from this study indicate the need for further research, which should be extended to other regions of Serbia. A wider population sample should be subjected to seroconversion studies, paying particular attention to individuals whose profession entails a risk of tick bites, due to prolonged time in the ticks' natural habitat. Constant monitoring of the principal reservoir and most important vector of *Borrelia*, the tick *I. ricinus*, as well as monitoring the factors that enable the completion of its natural life cycle, are significant measures in the prevention of Lyme borreliosis.

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Authors' contributions

V.S., D.M., M.M. and P.B. carried out the experiment. V.S., D.M., B.Č. and P.B. wrote the manuscript with support from S.T.

V.S., M.M., S.T., D.M. and P.B. collected the samples. S.T. and D.L. helped supervise the project. V.S. and S.T. conceived the original idea. V.S. and P.B. supervised the project.

Competing interests

Authors declare no conflict of interest.

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B. BURGDORFERI INFEKCIJA U KRPELJMA UKLONJENIH SA LJUDI I PRISUSTVO ANTITELA PROTIV BORELIJA KOD PACIJENATA INFESTIRANIH KRPELJIMA

SIMIN Verica, LALOŠEVIĆ Dušan, MIJATOVIĆ Dragana,
TOMANOVIĆ Snežana, MILJEVIĆ Milan, ČABRILO Borislav,
BOGDAN Ivana, BANOVIĆ Pavle

Kratak sadržaj

Glavni cilj ove studije je bio da se ispita prisustvo *B. burgdorferi* infekcije u krpeljma uklonjenih sa pacijenata radi utvđivanja lokaliteta sa povećanim rizikom za obolevanje od lajm borelioze, kao i ispitivanje prisustva IgM i/ili IgG antitela usmerenih protiv *B. burgdorferi* s. l. kompleksa u serumu pacijenata infestiranih krpeljima. Sa pacijenata je prikupljeno i zoološki ispitano 108 krpelja. PCR analiza krpelja korišćena je za utvrđivanje prisustva *B. burgdorferi* s. l. kompleks kod 91 krpelja od ukupnih 108. Za otkrivanje IgM i/ili IgG antitela protiv *B. burgdorferi* s. l. korišćeni su linijski rekombinantni imunoblot testovi. Najčešći identifikovani krpelj je *Ixodes ricinus*. *B. burgdorferi* s. l. je bila prisutna u 37 od 91 testiranog krpelja (40,7%). Prisustvo IgM/IgG antitela protiv *B. burgdorferi* s. l. otkriveno je kod 12 od 61 pacijenta (19,7%). Najviše inficiarnih krpelja je sa teritorije AP Vojvodine (11 opština), gde je urbani deo Novog Sada lokalitet sa najvećim brojem inficiranih krpelja – 6.

Ključne reči: *B. burgdorferi* sensu lato, *Ixodes*, lajmska borelioza, krpelj, seroprevalencija, Vojvodina