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First molecular detection of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected from humans in the Sarajevo Canton (Bosnia and Herzegovina)

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Borrelia burgdorferi sensu lato is a complex of spirochetes which includes five pathogenic species that cause Lyme borreliosis (LB), the most common tick-borne disease in humans (Stanek & Strle 2018). The Sarajevo Canton, as a part of the central mountain and basin area of the Bosnian Dinarides, is influenced by the Central European continental climate from the north and the Mediterranean climate from the south (Drešković & Đug 2006).

In total, 1081 cases of LB were reported in Bosnia and Herzegovina in the period from 2002 to 2018 according to the statistics of authorized public health institutions (https://www.zzjzfbih.ba/, https://www.phi.rs.ba). However, only one molecular study has been conducted in Bosnia and Herzegovina so far to test for the prevalence of *B. burgdorferi* sensu lato in free questing *Ixodes ricinus* (Linnaeus, 1758) (Hodžić *et al.* 2017). Although six samples showed to be positive for *Rickettsia monacensis*, none of 87 adult ticks gave positive findings for *Borrelia*. Spirochetes from *B. burgdorferi* sensu lato complex were detected and cultivated in Serbia and Croatia, the neighboring countries to Bosnia and Herzegovina (Ćakić *et al*. 2019; Rijpkema *et al.* 1996). According to this, positive findings of *Borrelia* are to be expected in ticks from our country as well.

This study aimed to utilize the nested polymerase chain reaction (nested PCR) to test for the presence of spirochetes from *B. burgdorferi* sensu lato complex in *I. ricinus* ticks removed from the patients in The Center for Emergency Medical Assistance of the Sarajevo Canton in period between March and June 2019. All samplings were done with the patients' consent.

Ticks were stored in separate tubes with 96% ethanol until further identification which was done following Estrada-Peña *et al*. (2004). Total genomic DNA was extracted using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Molecular identification of *I. ricinus* ticks was performed using primer pair dITS678 and rITS814 (Rumer *et al*. 2011). Nested PCR for detection of *B. burgdorferi* sensu lato targeting intergenic *rrf* (5S)–*rrl* (23S) region was performed using two primer pairs (Cerar *et al*. 2008; Postic *et al.* 1994). The first PCR was carried out according to Cerar *et al.* (2008) and the second PCR was performed according to Wang *et al.* (2014). In both PCRs, a positive AMPLIRUN *Borrelia burgdorferi* DNA control (Vircell, Spain) and a negative control were run in parallel with samples. PCR products were analyzed on 2% agarose gels. The sequencing of PCR products was

done in Macrogene Europe B.V. (Amsterdam, the Netherlands). Sequences were identified and analyzed using nucleotide BLAST tool at the National Centre for Biotechnology Information (https://blast. ncbi.nlm.nih.gov/Blast.cgi) and FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.ge os piza.com).

We received 50 tick samples removed from 50 patients (28 females and 22 males). Two samples did not have morphological characteristics of ticks and were excluded from further analysis. According to patients' statements, no ticks were subjected to any treatment before removal by medical staff. Based on age, patients were classified into four groups: (1) 0–18 (15 patients), (2) 19-35 (4 patients), (3) 36–65 (19 patients) and (4) +65 (12 patients). The number of ticks found per patient was one. Sixteen reported localities of possible tick infestation were registered in different parts of the Sarajevo Canton (Fig 1).

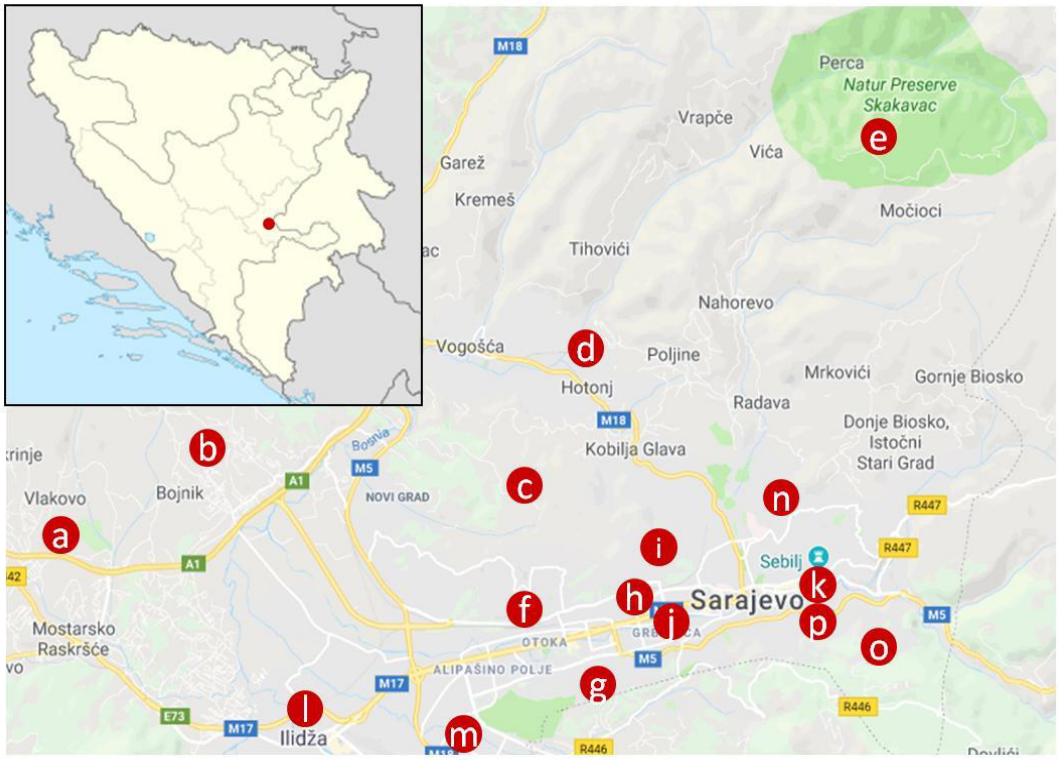

FIGURE 1. Localities of the tick bite in the Sarajevo Canton reported by patients: a—Vlakovo, b—Ahatovići, c—hill Žuč, d—Donji Hotonj, e—Waterfall Skakavac, f—Buća Potok, g—Švrakino selo, h—Pofalići, i—Velešići, j—Wilson's Promenade, k—Ferhadija, l—Ilidža, m—Vojničko polje, n—Sedrenik, o—Bistrik and Hrid, p—Čobanija

All 48 tested ticks were identified as *I. ricinus* using both morphological and molecular identification methods. Fourteen of them were determined as nymphs (29.20%) and 34 as females (70.80%). Ticks were classified according to the change of body volume after a blood meal as partially engorged midgut (diverticula filled with blood, volume changes of tick are not evident) (20, 41.60%), semi-engorged (volume changes of tick are evident) (14, 29.20%) and fully engorged (14, 29.20%).

Electrophoretic analysis of nested PCR products revealed that 32 of 48 tested ticks (66.70%) were positive on *B. burgdorferi* sensu lato, displaying the bands of 226–266 bp. Of all positively

tested ticks, one was nymph (3.10%) and 31 were females (96.90%). From all positively tested ticks, eleven (34.40%) were fully engorged and 21 were semi-engorged (65.60%), indicating a higher risk of *Borrelia* infection for patients (Hofhuis *et al*. 2017).

Two sequences were obtained from PCR-positive *I. ricinus.* Sequences were deposited in the GenBank database under the accession numbers MN510850 and MN510851. Sequence under accession number MN510850 showed 100% similarity to *rrs(5S)–rrf(23S)* sequences of *B. spielmanii* deposited in GenBank. Sequence under accession number MN510851 showed 99% similarity to *rrs(5S)–rrf(23S)* sequences of *B. lusitaniae* deposited in GenBank. The results of this study represents the first molecular detection of pathogenic species of *B. burgdorferi* sensu lato complex in ticks inhabiting the area of the Sarajevo Canton, as well as those species with potential pathogenic risk such as *B. lusitaniae*. The results provided preliminary insight into the presence of *Borrelia* in the studied area and represent the starting point for further molecular detection and determination of *B. burgdorferi* sensu lato species across the whole territory of Bosnia and Herzegovina.

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