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Monitoring of Hard Ticks at Urban Recreational Sites in the City of Zagreb from 2016 to 2018

Praćenje tvrdih krpelja u urbanim rekreacijskim odredištima grada Zagreba od 2016. do 2018. godine

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Abstract

As tick-borne disease incidence and distribution is increasing worldwide, there is a true need for better understanding of the distributional ecology of their vectors. The aim of this study was to determine the diversity of hard ticks fauna (Acari: Ixodidae) and their seasonal dynamics in different habitats at three urban recreational sites in Croatia's capital, the City of Zagreb, known as the natural foci of Lyme borreliosis and tick borne encephalitis. Within a three-year period (2016 - 2018), the only species detected was Ixodes ricinus Linnaeus, 1758. Using flag dragging method 506 ticks were sampled; 273 (54%) in their nymphal stage, 166 (33%) as larvae and 64 (13%) as adults. The highest abundance of ticks was recorded at forest habitat. Seasonal activity showed their peaks at midspring and midsummer. Continuous monitoring of hard tick population in urban areas should and could become a standard method of tick-borne diseases prevention.

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Sažetak

Kako se učestalost i rasprostranjenost krpeljnih bolesti širom svijeta povećava, postoji stvarna potreba za boljim razumijevanjem distribucijske ekologije njihovih vektora. Cilj ovog istraživanja bio je odrediti raznolikost faune tvrdih krpelja (Acari: Ixodidae) i njihovu sezonsku dinamiku na različitim staništima u tri urbana rekreacijska odredišta u glavnom gradu Hrvatske, Zagrebu, poznatom kao prirodnom žarištu lajmske borelioze i krpeljnog meningoencefalitisa. Tijekom trogodišnjeg razdoblja (2016. - 2018. god.) jedina determinirana vrsta bila je Ixodes ricinus Linnaeus, 1758. Metodom krpeljne zatege, prikupljeno je 506 jedinki tvrdih krpelja, od čega 273 (54%) u razvojnom stadiju nimfe, 166 (33%) u stadiju ličinke i 64 (13%) u odraslom stadiju. Najveći broj krpelja sakupljen je u šumskom staništu. Sezonska aktivnost krpelja dosezala je maksimume sredinom proljeća i sredinom ljeta. Kontinuirano praćenje populacije tvrdih krpelja u urbanim područjima trebalo bi, i moglo bi, postati standardna metoda prevencije bolesti koje prenose krpelji.

Introduction

Hard ticks (Acari: Ixodidae) are one of the main arthropod-borne disease vectors in Europe and also worldwide. Due to the recent predictions of their expansion, which includes the increasing incidence and distribution of tick-borne diseases in correlation to climate change, they manage to attain more of public attention[1-3]. Infesting a wide variety of wild vertebrate species, but also livestock, companion animals and humans, these obligatory hematophagous ectoparasites transmit numerous infectious agents (virus, bacteria, protozoa)[4, 5] causing the spread of various diseases like Lyme borreliosis (LB), tick-borne encephalitis (TBE), tularaemia, Mediterranean spotted fever, human granulocytic anaplasmosis etc.[6-9]. From the first studies on the occurrence of ixodid species in Croatia, about 80 years ago, till today, around 22 tick species, belonging to five genera (*Ixodes, Haemaphysalis, Rhipicephalus, Dermacentor, Hyalomma*) were recorded, and the most abundant and widespread species is the *Ixodes ricinus* (Castor bean tick, Sheep tick, Deer tick, Forest tick)[10, 11]. *Ixodes ricinus* plays an important role in spreading LB and TBE in Croatian inland [12-19], especially in the northern and northwestern part where high prevalence (40 - 90%) of *Borrelia burgdorferi* within tick population has been recorded [13, 20, 21]. Tick-borne diseases are characterized by seasonal occurrence with the highest rate of infection in the summer months[16] which is in correlation with tick's preferring warm and humid environmental conditions[22]. Being limited by temperature and humidity and also with the distribution of their hosts, ticks inhabit deciduous and mixed forests as a typical microhabitat (providing leaf litter that maintains high relative humidity), but also coniferous forests, grasslands, pastures some urban, peri-urban, and recreational environments with matching microclimate and enough host population[23-30]. The city of Zagreb, as Croatia's capital inhabited by nearly the forth of Croatian's population, has been known as natural foci of LB and TBE and also for high prevalence of *B. burgdorferi* within the tick population[13, 16]. Since ticks have specific preferences towards their microhabitat, being aware of the hotspots they inhabit while seeking for the next host, may help prevent the diseases. The aim of this study was to establish the structure and population dynamics of hard ticks in different habitats at three popular recreational urban sites in the City of Zagreb in period from 2016 to 2018.

Methods

This study was carried out at three recreational urban sites in Croatia's capital, the City of Zagreb, that is located in the northwest of the country, at the southern slopes of the mountain Medvednica along the Sava river (122 m above sea level). The first urban site was in the oldest public park in Zagreb; Park Maksimir (316 ha, 120 – 167 m above sea level, 11°C mean annual temp., annual precipitation 870 mm), located in the eastern central part of the city, with forest association of common oak (*Quercus robur*) and hornbeam (*Carpinus betulus*) (*Carpino betuli – Quercetum roboris* /Anić 1959/Rauš 1969) in southern lowland parts, and association of Sessile oak (*Quercus petraea*) and horn beam (*Epimedio – Carpinetum betuli* / Ht. 1938 / Borh. 1963) and also with Sweet chestnut (*Castanea sativa*) at elevated terrains (*Querco – Castanetum sativae* Horvat 1938). In Park Maksimir, besides forests, there are meadows, lakes and streams. The second urban site was recreational and sports centre (RSC) Jarun (240 ha), located in the southwestern part of Zagreb, on the northern banks of river Sava, with two lakes, six islands, meadows and groves with dominant autochthonous willow and poplar vegetation. The third urban site was RSC Bundek (55 ha) located in the southern part of the city, on the southern banks of river Sava, with two lakes, meadows and groves with dominant autochthonous willow and poplar vegetation. Ticks were sampled in all three parks at different habitats, which were chosen according to park landscape. This resulted in some overlapping as well as different habitats (Table 1.). At Park Maksimir ticks were sampled during years 2016, 2017 and 2018, and at RSC Jarun and RSC Bundek during 2017 and 2018.

Table 1. Habitat types at which ticks were collected at three recreational sites in Zagreb (2016 – 2018)

Locality	Habitat				
Park Maksimir (2016-2018)	Forest	Forest edge	Meadow	Forest trail	Lake embankment
RSC Jarun (2017-2018)	Forest	Forest edge	Meadow	Trail by the lake	Park area
RSC Bundek (2017-2018)	Forest	Pond edge	Meadow	River embankment	Park area

Tick were sampled by flag dragging method[31-33], using white flannel cloth (1m x 1m). Sampling was done once a month, from spring till autumn. Transects were of 50 m in length and sampling was done in 30-minute periods. Identification of ticks was done using standard keys for European ticks[34] via stereomicroscope (Olympus Leica Wild m28).

Results

From 2016 to 2018, 506 ticks were sampled at three popular recreational sites in Zagreb; 326 (65%) in Park Maksimir, 173 (34%) at RSC Jarun and seven (1%) at RSC Bundek. All sampled specimens were morphologically identified as *Ixodes ricinus* Linnaeus, 1758 (Wood tick, Deer tick, Sheep tick, Forest tick, Castor bean tick). At Park Maksimir ticks were sampled 22 times (7/2016, 9/2017, 6/2018), at RSC Jarun 17 times (9/2017, 8/2018) and at RSC Bundek 13 times (8/2017, 5/2018). The highest number of sampled ticks were in nymphalid stage (276; 54%), 166 (33%) were in larval stage and 64 (28 ♂, 36 ♀) (13%) were adults. Table 2. shows the number of collected ticks in their developmental stages collected at different sampling sites.

The highest number of ticks in Park Maksimir (N=237; 73%) and also at RSC Jarun (N=114; 66%) were sampled at forest habitat (Table 3.), while at RSC Bundek it was habitat river embankment (N=4; 57%) that showed the highest abundance of ticks (Table 3.). In total, for all three recreational sites, the highest tick abundance was recorded at forest habitats, (N=352; 69.6%), following forest edge habitat with 106 ticks (20.9%) (Table 3.), while other habitats had tick abundance under 3.2%.

From a total of 506 tick specimens sampled most were nymphs covering 54.5% (N=276) of all catch (Figure 1.). Tick larvae made 32.8% (N=166) and adults made 12.6% (N=64) of all catch. Similar ratio of adults, nymphs and larvae was found within Park Maksimir and RSC Jarun (Figure 1.).

Table 2. Number of ticks in their developmental stages, sampled at three recreational sites in Zagreb (2016 – 2018)

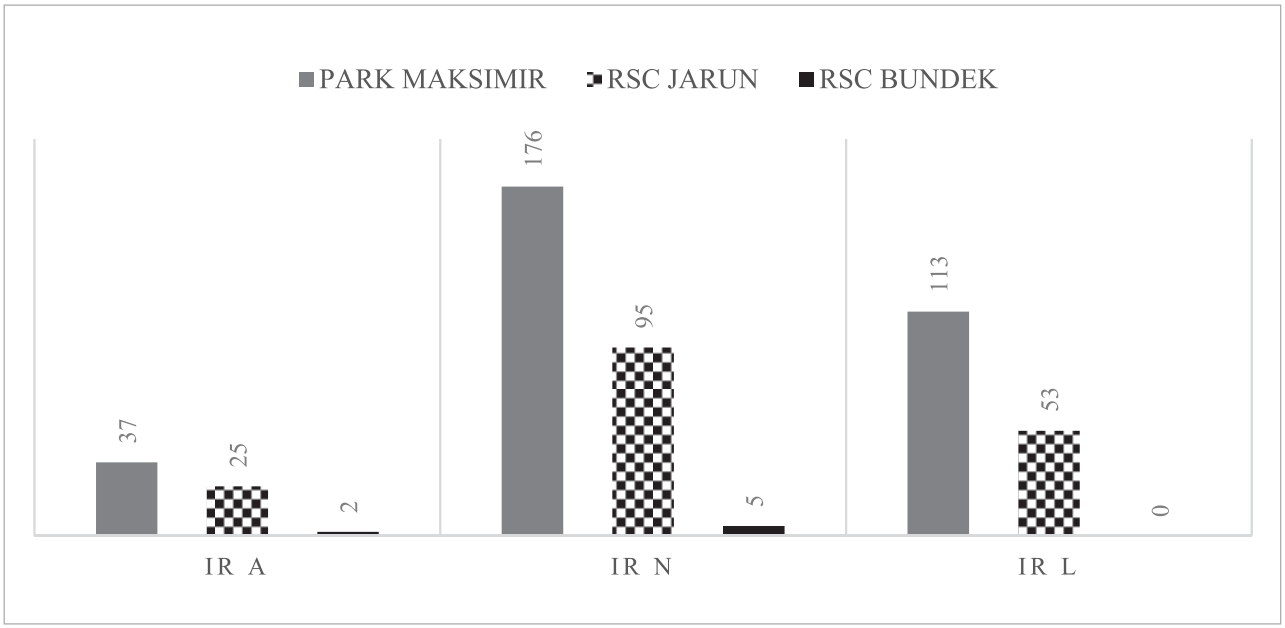
Locality	IR L	IR N	IR A [♂/♀]	Σ
Park Maksimir (2016-2018)	113	176	37 [12/25]	**326**
RSC Jarun (2017-2018)	53	95	25 [14/11]	**173**
RSC Bundek (2017-2018)	0	5	2 [2/0]	**7**
Σ	**166**	**276**	**64 [28/36]**	**506**

(IR= *Ixodes ricinus* Linnaeus, 1758; A=adult; ♂: adult male; ♀: adult female; N=nymph; L=larva)

Table 3. Abundance of sampled ticks (*Ixodes ricinus* Linnaeus, 1758) at five different habitats at three recreational sites in Zagreb (2016 - 2018)

Habitat	Park Maksimir (2016-2018	RSC Jarun (2017-2018)	RSC Bundek (2017-2018)	Σ
Forest	237	114	1	**352**
Forest edge	55	51	-	**106**
Forest trail	16	-	-	**16**
Meadow	12	3	0	**15**
Lake embankment	6	-	-	**6**
Park area	-	1	0	**1**
Trail by the lake	-	4	-	**4**
River embankment	-	-	4	**4**
Pond edge	-	-	2	**2**
Σ	**326**	**173**	**7**	**506**

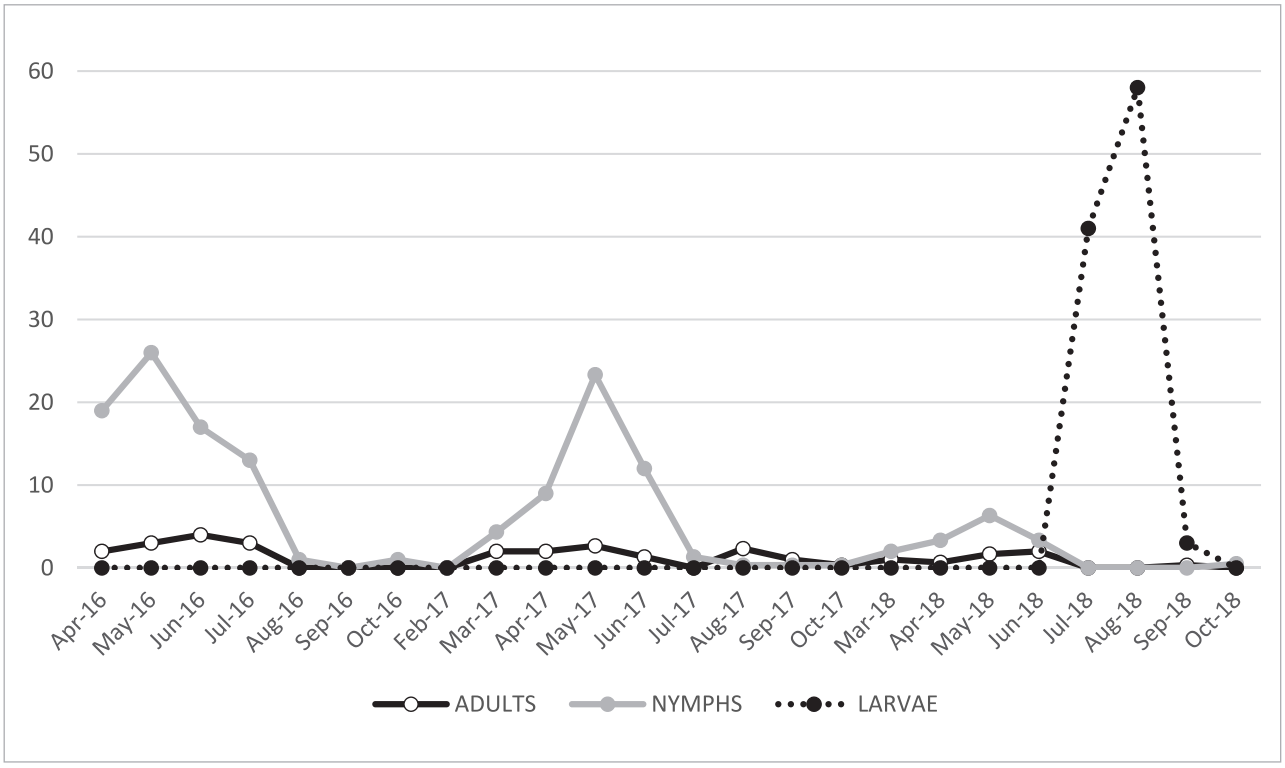
Figure 1. Different life stages of sampled ticks at three recreational sites in Zagreb (2016 – 2018)



(IR= *Ixodes ricinus* Linnaeus, 1758; A=adult; N=nymph; L=larva)

Data of seasonal tick dynamics combined for all three recreational sites showed distinctive peaks in May for nymphs during all three years (Figure 2.). Adult ticks were collected in much smaller numbers in comparison to nymphs but were also most abundant in May or June (Figure 2.). Larvae were collected only in the summer of 2018 peaking in August with highest abundance in comparison to adults and nymphs (Figure 2.).

Figure 2. Relative monthly tick (Ixodes ricinus Linnaeus, 1758) abundance for three life stages at all recreational sites in Zagreb (2016 – 2018)



Discussion and Conclusion

Most of the comprehensive studies on tick fauna in Croatia has been done from the 1950s till 1980s and the emphasis of those studies were on the distribution, dynamics and the diversity of tick fauna along the Adriatic coast and on the islands in the Adriatic Sea[10]. While collecting the data regarding the occurrence of ixodid species in Croatia, Krčmar (2012)[10] counted 79 sampling sites (only 16 in inland!) and also contributed with several new localities, mainly at eastern part of the country[35]. Considering that the City of Zagreb is known as a natural focus of Lyme borreliosis and tick-borne encephalitis[13, 20, 21], there is not much information about species distribution, abundance or seasonal dynamics of hard ticks in the Croatia's capital. From 1959 till 1975 in Zagreb and its vicinity, only three species from genus *Ixodes* were recorded (*Ixodes ricinus* Linnaeus, 1758; *Ixodes arboricola* Schulze & Schlottke 1930; *Ixodes frontalis* Panzer, 1798)[36-38] and the list was expanded in 2008 with one species from genus *Dermacentor* (*D. reticulatus* Fabricius, 1794) [13]. In this study, *Ixodes ricinus* was the only species of hard ticks collected at three popular urban recreational sites in Zagreb. These results agree with previous findings of *Ixodes ricinus* being the most widely distributed species in continental part of Croatia, as it is in Europe[10, 39], but also indicates a little diversity of tick fauna in the City of Zagreb. Through our research no *Dermacentor reticulatus* was found, although it has been previously sampled, using the same sampling methods, at RSC Jarun in 2008[13]. Except the slow natural succession of forest habitat, no major changes in the environment, nor the vegetation management, or the presence of potential hosts, took place at RSC Jarun since 2008, to our knowledge. Including more micro localities in future monitoring of ticks at RCS Jarun could provide some answers. Our work is in line with the general trend of collecting *Ixodes ricinus* more frequently than the other species during field sampling[40]. In every locality included in this study, most ticks were collected in their immature and more abundant, nymphal stage, in which they, being physically smaller and harder to notice, pose a bigger threat to humans regarding transmission of different pathogens (Table 2.)[41]. In Park Maksimir and in RSC Jarun, the habitat with the highest abundance of ticks, in all developmental stages, was the forest type habitat and that matches tick's microclimate preferences (Table 3.) [23-26, 42]. The second habitat with the highest number of sampled ticks was the forest edge. Ninety percent of sampled ticks in Park Maksimir and 95% at RSC Jarun were collected in forest and forest edge, indicating that the suitability of habitats for *Ixodes ricinus* is highly influenced by forest cover in urban areas providing ticks with adequate microclimate and reproductive hosts (27-30). Comparing all three localities included in this research Park Maksimir, although not inhabited anymore by large herbivorous, like European roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and even carnivores like grey wolf (*Canis lupus*), still provides a home for largest number of animal species (e.g. red squirrel; *Sciurus vulgaris*, edible dormouse; *Glis glis*, marten; *Martes ssp*., red fox; *Vulpes* and over 100 bird species) that make potential ticks hosts. Another thing that makes Park Maksimir a suitable habitat for stable tick populations is its' sessile oak forest communities with ferns (*Pteridium aquilinum)* in under tree cover[34]. At RSC Bundek only seven specimens of ticks were collected during two-year period and all three habitats on which they were collected included tree coverage to some extent (Table 3.). The main reasons for such a small number of ticks at RSC Bundek is, most presumably very good vegetation management (mowing once a week) along with an absence of tick's natural hosts, while Bundek, as a recreational site is in highly urban area (43) surrounded by heavy traffic roads and residential buildings. Unfortunately, there is a lack of information about small rodent populations at these three sites, while no continuous monitoring is being conducted. Seasonal dynamics of sampled ticks at Park Maksimir showed typical peak during spring time, in May, which corresponds to previous findings[44], but it also reached its second peak in the mid-summer, in August, which is a bit earlier than is usually expected[42]. At RSC Jarun, the peaks were recorded in May 2017 and in July 2018. At RSC Bundek the autumn peak was recorded in October 2017 and according to Estrada-Peña et al.[34] that corresponds to activity of adult ticks. In Park Maksimir and RSC Jarun, ticks in their nymphalid stage, were most abundant in May, while as larvae they were sampled only in the midsummer of 2018 (Figure 3.) which corresponds to different field studies[34] and also the results of recent study in the surroundings of city Beli Manastir, in eastern Croatia[35]. At all three localities ticks in their adult stage were collected till midsummer in July, when they are usually affected by diapause[40]. Seasonal dynamics at different habitats (Table 3.) indicates that in opened habitats which are more exposed to direct sunlight (like meadows), ticks will appear from early spring till early summer and then again at the beginning of autumn, and in forest habitats they'll find convenient temperature and humidity conditions even in midsummer[45]. We have collected specimens of *Ixodes ricinus* in all three life stages and it matches a fact that all stages of this species climb on vegetation in search for a

host[34]. Despite extremely warm thermal conditions in the City of Zagreb in 2016, 2017 and 2018[46], the number of sampled ticks was increasing through the years in Park Maksimir and at RSC Jarun and it is in line with *Ixodes ricinus* being less sensitive on temperature changes[47] than the other tick species. Stability of the population was presumably aided by the annual precipitation[40, 48, 49], that was in line with multi-annual average during this three-year period[47]. There are already many indications that climate change will back up the expansion of ixodid ticks[50, 51] and it will also include the increasing incidence and distribution of tick-borne diseases[1-3, 45, 52]. While *Ixodes ricinus*, with its ecological plasticity and ability to adapt, will continue to pose a threat to human health[53-55], the continuous monitoring, detection and reporting of its population, and also of the population of its main hosts, would surely help to improve public health standards by means of prevention.

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