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HARD TICK FAUNA (ACARI: IXODIDAE) IN DIFFERENT TYPES OF HABITATS IN THE CITY OF OSIJEK (EASTERN CROATIA)

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A faunistic study of hard ticks was conducted from February 2019 to August 2019 in seven different habitats in the city of Osijek. In all, 664 specimens were collected and classified into 2 species and 2 genera. *Ixodes ricinus* (Linnaeus, 1758) is the most abundant species in the studied area with 493 specimens, followed by *Haemaphysalis concinna* Koch, 1844 with 171 specimens collected. The largest number of ticks was collected in July, in the larval stage. Statistically significant differences were observed in the number of specimens with respect to different habitat types and sampling months ($p < 0.05$). The data collected constitute a pilot study of the hard tick population in the city of Osijek, the largest city in eastern Croatia, which is characterized by the largest number of green spaces and parks in Croatia.

Key words: hard ticks, *Ixodes ricinus*, *Haemaphysalis concinna*, Osijek, Croatia

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Istraživanja faune tvrdih krpelja provedena su od veljače do kolovoza 2019. godine na sedam različitih stanišnih tipova unutar grada Osijeka. Prikupljene su 664 jedinke, svrstane u 2 vrste i 2 roda. Vrsta *Ixodes ricinus* (Linnaeus, 1758) je najbrojnija vrsta na istraživanim lokalitetima s 493 skupljene jedinke, te slijedi *Haemaphysalis concinna* Koch, 1844 sa 171 jedinkom. Najveći broj krpelja skupljen je u srpnju, u razvojnom stadiju larve. Zabilježene su statistički značajne razlike u brojnosti jedinki uzorkovanih vrsta krpelja u odnosu na različite tipove staništa i mjesec uzorkovanja ($p< 0,05$). Skupljeni krpelji predstavljaju nulto stanje populacije tvrdih krpelja na području grada Osijeka, najvećeg grada istočnog dijela Hrvatske, grada s najviše zelenih površina i parkova u Hrvatskoj.

Ključne riječi: tvrdi krpelji, *Ixodes ricinus*, *Haemaphysalis concinna*, Osijek, Hrvatska

INTRODUCTION

Within the group of arthropods, ticks are gaining increasing attention because of their vector capabilities and the transmission of human and animal pathogens, including protozoa, viruses, bacteria, fungi and various types of infectious diseases (Goodman *et al.*, 2005). This is precisely what makes them a worrying group of arthropods, which transmit more pathogens worldwide than any other group. Their growth and development are facilitated by the blood of the host (Durden, 2006). Tick bites can cause toxic reactions, allergic responses, even paralysis, and the resulting wounds can be potential entry points for secondary microbial infections (Haddad *et al.*, 2018). Glo-

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bally, they are the most important vectors in the spectrum of veterinary interests and the second most important after mosquitoes in the public health sector (de la Fuente *et al.*, 2008; Mrljak *et al.*, 2017). Tick-borne zoonoses have been known and studied since the second half of the 19th century (Hoogstraal, 1967). However, over the last two decades, there has been an increase in the number of newly discovered diseases and the number of reported cases of patients (Pfäffle *et al.*, 2013). Despite improvements in prevention and treatment, tick-borne encephalitis is the most common tick-transmitted disease in Central and Eastern Europe and Russia (Amicizia *et al.*, 2013). In the European Union between 2012 and 2016, 23 countries reported 12,500 tick-borne encephalitis cases of which 93% were confirmed cases, while 7% were probable cases (Beauté *et al.*, 2018). The annual notification rate fluctuated between 0.41 cases per 100,000 inhabitants in 2015 and 0.65 in 2013 in the European Union and the European Economic Area (Beauté *et al.*, 2018). Also, in Germany infection with tick-borne encephalitis virus showed a slight but significantly rising temporal trend from 2001 to 2018. Risk areas increased from 129 districts in 2007 to 161 in 2019 (Hellenbrand *et al.*, 2019). In Croatia on average of 20 human cases of tick-borne encephalitis are reported each year, i.e. 0.26-1.05 cases per 100,000 inhabitants (Erber & Vuković-Janković, 2020). Environmental factors in addition to sociodemographic changes, agrarian development, deforestation and other anthropogenically influenced modifications of ecological systems greatly affect the dynamics of tick populations and the spread of pathogens (Estrada-Peña & de la Fuente, 2014). Therefore, interdisciplinary collaboration is needed to clarify changes in tick distribution. Data on hard tick fauna in eastern Croatia are rather scarce and reported in several published papers (Krčmar, 2012; Krčmar *et al.*, 2014; Krčmar, 2019). In recent studies on eastern Croatia, seven hard tick species from the genera *Ixodes*, *Dermacentor* and *Haemaphysalis* were recorded at 48 localities (Krčmar, 2019), but the area of the city of Osijek was omitted from that study. Osijek is the largest city in eastern Croatia, with the largest number of green spaces and parks in Croatia; 17 parks with a total area of 394,000 m^2. Therefore, the city of Osijek is an important demographic, industrial and cultural center, whose green spaces need to be monitored and recorded for the presence of ticks to prevent the emergence and spread of associated pathogens. Hence, the aim of this study was to investigate hard tick fauna diversity and the abundance of ticks in seven different types of habitats in Osijek.

MATERIALS AND METHODS

Study area

The study was conducted in seven different types of habitats within the city of Osijek. Woodland habitats as well as meadows are located on the periphery of the city, while there are recreational parks and dog parks in the city perimeter. Due to human activity and high dog density within the urbanized center, these areas are particularly suitable for sampling.

The first locality is King Tomislav Park (45°33'40.23''N 18°41'29.51''E), classified as a Monument of Park Architecture. There are 83 taxa of shrubs and trees inside the park, such as common holly (*Ilex aquifolium* L.), field maple (*Acer campestre* L.) and eastern white pine (*Pinus strobus* L.).

The second locality is the flood defence embankment of the Drava River (45°33'44.11''N 18°42'56.77''E) - mesophilic meadows where the possibility of sampling largely depends on the water level of the Drava River and its maximums in water levels and flows during spring/early summer and fall.

The third locality is a riparian woodland near Osijek Zoo (45°34'7.69''N 18°41'20.68''E) located on the left bank of the Drava River. The study area is a type of willow and poplar flood woodland, however, due to the flood defence embankment along the left bank, the woodland is above groundwater level.

The fourth locality is a mesophilic meadow near the stadium Gradski vrt (45°32'35.03''N 18°41'36.04''E). It is located on the periphery of the city just a few meters from the stadium and residential units along the southern bypass of Osijek.

The fifth locality is white willow and poplar riparian woodland near the Pampas shooting range (45°34'5.94''N 18°38'11.25''E). It is surrounded by thoroughfares, residential areas and shopping centers.

The sixth locality is the Garo dog park (45°33'12.37''N 18°41'53.99''E) and the last and seventh locality is the Toti dog park (45°33'29.42''N 18°41'55.82''E) which are mowed relatively regularly and are quite frequently visited by dogs and their owners.

In the study area a continental climate prevails with an average annual rainfall of 700 mm to 800 mm, while the average air temperature is 10.7˚C (data from the Croatian Meteorological and Hydrological Service).

Tick collection

Hard tick sampling was performed over a seven-month period, from February to August 2019 with two field trips per month, with average sampling intervals of 15 days. It is important to keep an eye on the weather as it is essential to sample the ticks during dry and sunny days without precipitation.

Ticks were collected by the dragging-flagging method. Each sampling lasted 30 minutes per locality. Each sampling transect was 30 m long. All collected ticks were preserved in 96% ethanol solution. Identification and nomenclature followed that of Hillyard (1996) and Estrada-Peña *et al*. (2004).

Data visualization and analysis

Data were stored and analyzed in Excel and in programming language R (R Core Team, 2018) and because of the non-Gaussian distribution of the data tested by the Shapiro-Wilk normality test ($p < 0.05$); a non-parametric χ^2 test was applied to conclude what the data differences were and ultimately a post-hoc χ^2 test to determine between which samples statistically significant differences occurred (Agresti, 2007). Furthermore, the Sørensen index was used to describe and compare the similarity of the two samples within different habitat types (Durbešić, 1988).

RESULTS AND DISCUSSION

The processing and analysis of ticks collected in the area of the city of Osijek from February 2019 to August 2019 reveal the presence of 2 species, *Ixodes ricinus* (Linnaeus, 1758) and *Haemaphysalis concinna* Koch, 1844 in collected samples within 664 specimens. The most abundant species is *I. ricinus* with a prevalence of 74.25% (493 specimens), while the species *H. concinna* accounts for 25.75% (171 specimens) (Fig. 1).

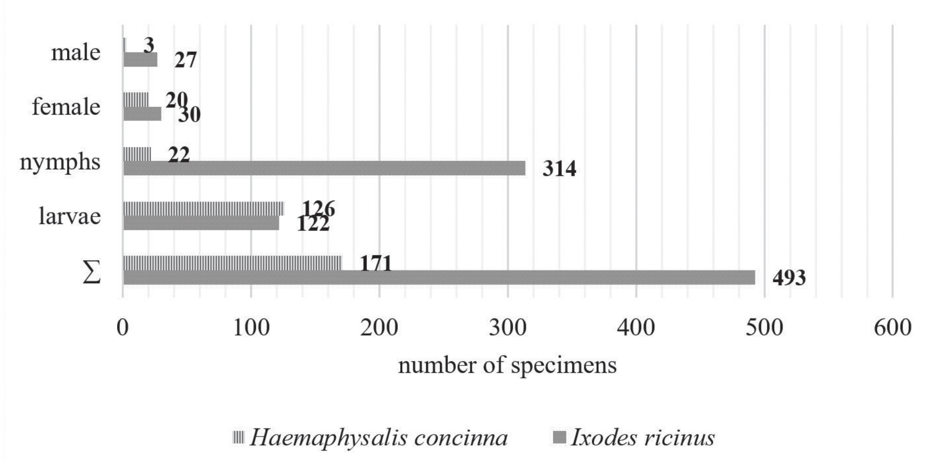

Fig. 1. Number of ticks classified by species and developmental stage sampled in the city of Osijek from February to August 2019.

The abundance of specimens in the nymphal stage is dominant, followed by the larval stage in the study area. The smallest abundance of sampled ticks is recorded in the adult stage, especially males (Fig. 1). Normally, adults and nymphs of the *I. ricinus* (common tick) are active between March or early April until early November for this climate zone (Dautel *et al.*, 2008). However, 16 active nymphs and 2 adults (1♂ and 1♀) of *I. ricinus* were collected and determined during February (Tab. 1). The activity of nymphs and adult ticks during the winter has also been observed in the forest habitats of the city of Berlin (Dautel *et al.*, 2008). The premise for the early activity of *I. ricinus* is climate change and the trend of mild winters (Gray *et al.*, 2009). Also, climate change contributes to the spread of *I. ricinus* in areas that were previously unsuitable for this tick species such as the northernmost areas of Europe and northern Eurasian areas (Dantas-Torres, 2015). During 2018 no significant deviations were observed in the amount of precipitation in this area, however, an air temperature anomaly of 2.0 °C was registered (Tab. 2). Likewise, the mean air temperature in February deviated from the average by 2.5 °C (Tab. 2). Accordingly, it would be advisable to abandon the established practice of tick sampling from March to November, because, according to the data collected so far, their activity during the winter is expected and therefore increases the risk of tick-borne zoonoses. The seasonal activity of *I. ricinus* is characterized by two abundance peaks (in spring from March to May and in July), while the highest activity of *H. concinna* is recorded in May and July (Fig. 2). Adults and nymphs reach their activity peak in spring and early summer in northern and central parts of Europe, whereas in the case of larvae, it is in mid-summer (Gray *et al.*, 2016). Also, in southern Italy, during summer very few nymphs and adult ticks of *I. ricinus* were recorded (Dantas-Torres & Otranto, 2013). These data correspond with the data obtained in this study, where the highest activity in the nymphal and adult stages were recorded during May, while highest activity of the larval stage was recorded in July (Fig. 3). Further, no specimens of *H. concinna* were sampled until mid-April 2019 (Tab. 1), as expected since nymphs and adults of this species are active from April/May in Central Europe (Rubel *et al.*, 2018). What deviates from the rule is the highest adult activity of

H. concinna in July in Osijek (Fig. 3). Similarly, the highest activity of *H. concinna* in Hungary occurred from April to July (Földvári & Farkas, 2005), although the activity is common throughout June for these areas (Rubel *et al.*, 2018). The reason for this is the abrupt change in air temperature late in May (air temperature anomaly -3.1 °C) and June (air temperature anomaly 3.0 °C) (Tab. 2) and probably the treatment of mosquitoes at the end of June. For the same reason, the total number of specimens collected in the month of June is much lower. The number of ticks collected in April is lower than in March (Tab. 1). The cause of the decreased number of collected specimens is inclement weather and frequent rainfall during April. χ^2 test and *post-hoc* χ^2 test show a statistically significant difference between the frequencies in most tick samples depending on the sampling month (χ^2=251.67, df=6, p<0.001).

Tab. 1. The number of collected hard ticks at seven sampling locations within the city of Osijek. Legend: ♀ (female), ♂ (male), n (nymph), l (larva).

Species/ Months	II	III	IV	V	VI	VII	VIII
King Tomislav Park							
Ixodes ricinus (Linnaeus, 1758)	0	0	1♂	0	0	0	0
∑	0	0	1	0	0	0	0
The flood defence embankment of the Drava River							
Haemaphysalis concinna Koch, 1884	0	0	1♀	0	0	0	0
∑	0	0	1	0	0	0	0
Woodland near Osijek Zoo							
Ixodes ricinus (Linnaeus, 1758)	1♂, 1♀, 16n	6♂, 5♀, 62n	1♂, 6♀, 57n	2♂, 2♀, 90n	1♂, 28n, 2l	1♂, 3♀, 15n, 64l	2n, 14l
Haemaphysalis concinna Koch, 1884	0	0	2♀	7♀, 2n, 7l	2♀, 9l	1♂, 3♀, 9n, 105l	1♂, 4n, 2l
∑	18	73	66	110	42	201	23
Woodland near Pampas shooting range							
Ixodes ricinus (Linnaeus, 1758)	0	30n	1♂, 3♀, 6n	2♂, 4♀, 3n	4♂, 2♀	3l	39l
Haemaphysalis concinna Koch, 1884	0	0	1♀, 1n	1n	3l	1♂, 4♀, 2n	3n
∑	0	30	12	10	9	10	42
Garo dog park							
Ixodes ricinus (Linnaeus, 1758)	0	0	1♂, 2n	6♂, 4♀, 3n	0	0	0
∑	0	0	3	13	0	0	0
Mesophilic meadow near the Gradski vrt stadium							
∑	0						
Toti dog park							
∑	0						

Tab. 2. Air temperature and precipitation amount anomalies in 2018 and 2019 in the city of Osijek (data from Croatian Meteorological and Hydrological Service).

Year	2018	2019						
Months		II	III	IV	V	VI	VII	VIII
Air temperature anomalies (°C)	2.0	2.5	2.6	1.0	-3.1	3.0	0.6	2.1
Precipitation amounts (percentile)	50	39	3	76	98	72	58	68
Category	extremely warm/ normal	warm/ normal	warm/ very dry	normal/ wet	very cold/ very wet	very warm/ normal	normal/ normal	very warm/ normal

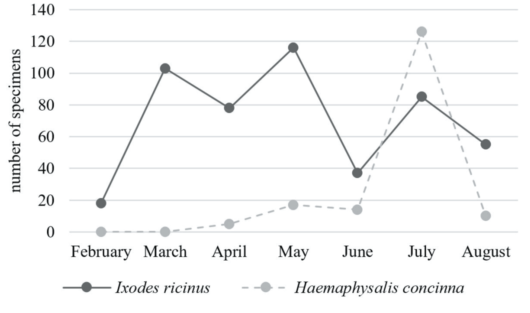

Fig. 2. Seasonal activity of hard ticks recorded in the city of Osijek from February to August 2019.

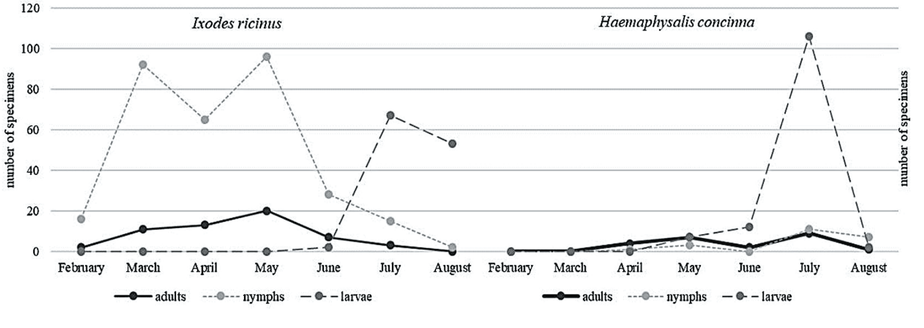

Fig. 3. Hard tick abundance in the city of Osijek from February to August 2019.

Forest habitats, with the largest number of sampled specimens (646 specimens, 97.28%), stand out in the qualitative and quantitative composition of the tick fauna, precisely because of the larger number of suitable hosts. Within the parks, the largest number of collected ticks was in the Garo dog park. On the other hand, not a single tick was collected in the meadow near the Gradski vrt stadium or in the Toti dog park (Tab. 1). χ^2 test and *post-hoc* χ^2 test show a statistically significant difference between the frequencies in most of the tick samples depending on the sampling site (χ^2=1573.3, df=4, p<0.001), except between samples collected in King Tomislav Park and the flood defence embankment of the Drava River (p=1.00000). The Sørensen index values indicate the highest faunal similarity of the sampled tick species between the woodland areas (100%). Similarly, the greatest similarity was observed between King Tomislav Park and Garo dog park (100%). On the other hand, the similarity between King Tomislav Park and the woodland near Osijek Zoo is 66%. Also, the same index value was recorded in the comparison of King Tomislav Park and the woodland near the Pampas shooting range. In addition, the woodland near Osijek Zoo and the woodland near the shooting range share a 66% similarity with the flood defence embankment of the Drava River and the Garo dog park. After the species *I. ricinus* and *Dermacentor reticulatus*, *H. concinna* is the third most abundant species in Central Europe (Rubel *et al.,* 2018). In numerous studies, the share of *H. concinna* specimens among the ticks collected is usually around 2% (Uspensky, 2014). For example, a study conducted in Poland recorded 1,600 collected ticks, of which 2.7% belonged to *H. concinna* and 95% to *I. ricinus* (Kiewra *et al.*, 2019). Additionally, in Slovakia, 1.3% of *H. concinna* and 98.6% of *I. ricinus* were collected (Kazimírová *et al.*, 2016). Generally, among all European hard tick species, *I. ricinus* was often recorded as the most abundant species in local tick fauna (Estrada-Peña & Venzal, 2006). However, southward to Hungary the result was similar to the results of this study. The share of *H. concinna* was 17.8% and 78.6% of *I.ricinus* (Hornok *et al.*, 2014). This indicates the widespread distribution of the species *H. concina*, a vector of various pathogens and agents of diseases, including Lyme borreliosis, tick-borne encephalitis virus, *Rickettsia sibirica*, *Coxiella burnetii* and Crimean-Congo hemorrhagic fever (Meng *et al.*, 2016). The collected samples contained many *H. concinna* specimens (171 specimens), but not a single specimen of the species *D. reticulatus*, despite the high prevalence recorded in various habitats of Central Europe, including alluvial forests, wetlands, dry habitats and urban areas (Rubel *et al.*, 2016). The small diversity of tick fauna in the area of the city of Osijek is not so surprising precisely because of the lack of tick species diversity in the larger urban areas of Croatia, such as the city of Zagreb. Within the different habitats at three popular recreational urban sites in the city of Zagreb in the period from 2016 to 2018, only one tick species, *I. ricinus*, was identified (Vucelja *et al.,* 2019). Also, *I. ricinus* was the most common in urban areas of Germany, Poland, the Czech Republic and some other countries as well (Uspensky, 2014). Due to its wide distribution and large abundance *I. ricinus* is considered the most important vector of many zoonotic pathogens of viral and bacterial origin (TBEV, *Anaplasma phagocytophilum*, *Borelia burgdorferi s.l.*, *Francisella tularensis*) in Croatia (Borčić *et al.*, 1978; Jemeršić *et al.*, 2014; Huber *et al.*, 2017; Mrljak *et al.*, 2017), as well as in many areas of Europe (Furness & Furness, 2018). The results of this study in the city of Osijek were confirmed by a recently published study in Vienna (Vogelgesang *et al.,* 2020).

CONCLUSIONS

Due to their ability to transmit numerous pathogens and diseases, tick populations need to be continuously monitored and controlled at local and national level. Many hard-tick species, including *I. ricinus* and *H. concinna*, are well adapted to existing conditions in urban areas and thus pose a constant threat to human and domestic animal health. It is important to emphasize that the distribution of *H. concinna* is not monitored as a part of the VectorNet project (ECDC, 2019), despite its high prevalence in many large cities in Europe. Therefore, this species should be considered as a relevant species for risk assessments.

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