Diversity, ecology, and seasonality of hard ticks (Acari: Ixodidae) in eastern Croatia

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ABSTRACT: The diversity of hard tick fauna was studied in different forest communities in 48 localities of eastern Croatia using the dragging-flagging method and by hand from ten different vertebrate hosts. A total of 2,225 specimens comprising seven species in three genera were identified. *Ixodes ricinus,* the most abundant species (72.8% of all collected specimens) was recorded in 44 localities, followed by *Dermacentor reticulatus* in 19 localities, while the other five species (*D. marginatus*, *H. concinna*, *H. inermis*, *I. canisuga,* and *I. hexagonus*) were recorded in fewer localities. The highest ratio (67% of collected ticks) was in the adult stage with the predominance of females. The numbers of collected females, males, and nymphs of *I. ricinus* and *H. concinna* differed significantly. Ten new tick-host associations in Croatia were recorded. Fifty-three animals were infested with one tick species. Single species infestation with *I. ricinus* was found in 45.8% of animals, followed by *D. reticulatus* with 25.4%, *D. marginatus* with 10.2%, *H. inermis* and *I. hexagonus* with 3.4%, and *I. canisuga* with 1.7%. Coinfestation with two species of ticks were recorded in six animals. *Ixodes ricinus*, *H. inermis,* and *D. reticulatus* showed bimodal seasonal activity; for other species unimodal activity patterns were recorded. ***Journal of Vector Ecology* 44 (1): 18-29. 2019.**

Keyword Index: Ticks, Ixodidae, diversity, seasonality, hosts, Croatia.

INTRODUCTION

Hard ticks (Acari: Ixodidae) are very important vectors of animal and human disease agents, including viruses, bacteria, protozoa, and helminths of human and animal disease (Estrada-Pena and Jongejan 1999, Orkun et al. 2014). They are the second most important group of medically important hematophagic arthropods after mosquitoes (Jaenson and Jensen 2007, de la Fuente et al. 2008). Wild animals have a great impact on the epidemiology of tick-borne diseases, potentially as reservoirs or amplifying hosts for some pathogens (Lorusso et al. 2011, D' Amico et al. 2017). The main factors for the emergence and spread of ticks and their pathogens are connected by a series of human activities, including habitat changes, deforestation, globalization of the economy, international animal movements, urbanization, and climate change (Harrus and Baneth 2005, Mrljak et al. 2017).

The impact of climate change in Europe on the diversity of tick fauna was evidenced by the increased density and ability of ticks to spread into new areas, such as *Rhipicephalus sanguineus*, moving from the Mediterranean Basin to more northerly latitudes, or *Dermacentor reticulatus* in some areas of western Europe (Beugnet and Chalvet-Monfray 2013). It may be that warmer winters and extended autumn and spring seasons will continue to drive the expansion of the distribution of some tick species (e.g., *D. reticulatus, Ixodes ricinus, R. sanguineus*) to northern latitudes and to higher altitudes (Beugnet and Chalvet-Monfray 2013, Dantas-Torres 2015). Climate change will likely increase the climate niche of *I. ricinus*, including northernmost areas of Europe as well as northern Eurasian areas that were previously unsuitable for this species (Dantas-Torres 2015). The importance of tick-borne diseases is increasing all over the world and there is a need for the constant updating of data on the presence, distribution of ticks, and tick-transmitted diseases (Kiewra and Czulowska 2013, Mrljak et al. 2017). In some areas of southeastern Europe, ecological studies on the diversity of tick fauna are absent or were only sporadically performed. Systematic studies of tick fauna in Croatia were carried out only along the Adriatic coast and on islands in the Adriatic Sea during the second half of twentieth century (Mikačić 1961, 1963, 1965, Tovornik 1980, Tovornik and Brelih 1980, Tovornik and Vesenjak-Hirjan 1988). Also, in that period, some research was focused on the northwestern part of Croatia (Mikačić 1968, 1969, Borčić et al. 1978). These studies have resulted in the recording of 21 hard tick species from five genera. Despite a significant number of identified tick species in Croatian fauna, many areas in Croatia have not yet been sufficiently studied, including eastern Croatia. Indigent data on the hard tick fauna in eastern Croatia have prompted the initiation of this research with the aim of significantly contributing to a better knowledge of tick diversity, ecology, and seasonality in this region.

MATERIALS AND METHODS

Study area

Croatia is divided into four geographical regions: Eastern, Central, Alpine, and Mediterranean. The study area (Eastern Croatia) lies between the Drava River in the north, Sava on the south, Danube River in the east, and mountains (Psunj, Ravna gora, Lisina) in the west. This area is characterized by a moderately warm, humid climate with hot summers and expressed continentality during winters. The autochthonous forest flora belongs to pedunculate oak and hornbeam forests

(ass. *Carpino betuli – Quercetum roboris „typicum"*Rauš 1973) and 35% of forest communities in eastern Croatia belong to this forest (Vukelić et al. 2008). Hovever, in some parts of the study area, pedunculate oak and hornbeam forests are often in highly degraded stands. Pedunculate oak (*Quercus robur*) is the typical species of the upper canopy layer, but in some forest habitats, pedunculate oak has been replaced with black locust (*Robinia pseudoacacia*). On moist soils, out of the reach of flood waters, are the black and white poplar forests (ass. *Populetum nigrae – albe* Slavnić 1952). The dominant species of this two-layered canopy are black poplar (*Populus nigra*) and white poplar (*Populus alba*), while white willow (*Salix alba*) occurs with lower frequency. The alluvial pedunculate oak and dyers green weed forests are located on the partially flooded grounds. The autochthonous forests and the surrounding meadow offer the ideal living conditions for many animals that might serve as tick hosts. The study was carried out at 48 localities in the area of eastern Croatia, which covers 34 fields on the UTM grid (Figure 1, Table 1).

Sampling methods

Hard ticks were sampled using the dragging-flagging method and by hand from living domestics and dead wild animals. Samplings were carried out from the beginning of February to mid-December, 2016 and 2017, and in 2018 from February to August. Ticks were sampled two times per month during the spring and one time per month during the summer, autumn, and winter. Each sampling lasted 30 min per locality. Length of the transect on which ticks were sampled was from 25 to 30 m, using a white flannel 1m^2 flag.

Most samplings were carried out in the pedunculate oak and hornbeam forests, followed by black locust forests, poplar forests, and alluvial pedunculate oak and dyers green weed forests (Table 1). The more infrequent samplings were done on open pastures with 28 domestic animals: one horse (*Equus caballus*, L., 1758), five sheep (*Ovis aries* L., 1758), seven cats (*Felis catus*, L., 1758), and 15 dogs (*Canis lupus familiaris*, L., 1758) were inspected for the presence of ticks. Nineteen wild animals were examined: one stone marten (*Martes foina* Erxleben, 1777), one wildcat (*Felis silvestris*, Schreber, 1777), three European hares (*Lepus europaeus* Pallas, 1778), four red foxes (*Vulpes vulpes* L., 1758), five hedgehogs (*Erinaceus roumanicus*, Barrett-Hamilton, 1900), and five European badgers (*Meles meles* L., 1758) were inspected for the presence or absence of ticks. Fifteen shot red deer (*Cervus elaphus* L., 1758) and 15 wild boars (*Sus scrofa* L., 1758) were examined for tick presence. Samplings were made during daylight hours, mainly between 09:00 and 16:00. All collected ticks were put into plastic vials and preserved in 96% ethanol. The first half of the collections were stored in the Croatian Veterinary Institute in Zagreb, while the other half was stored in the Croatian Institute of Public Health in Zagreb with the aim of further research on the presence of certain pathogens in ticks.

Tick identification and analysis

To determine species, sex, and stage, a stereo-microscope and available identification keys were used (Hillyard 1996, Estrada-Peña et al. 2004, Hornok et al. 2017). Diversity of the tick fauna among different forest communities was measured
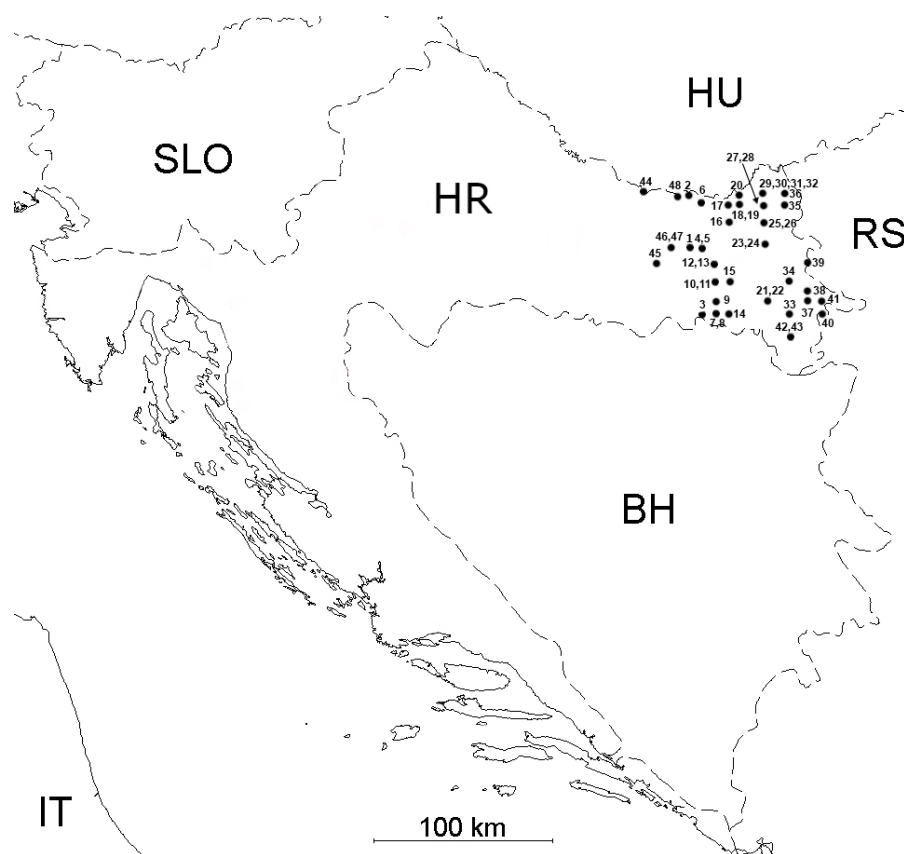

Figure 1. Sampling sites of hard ticks (Ixodidae) in eastern Croatia. Black circles from 1 to 48 are sampling sites in eastern Croatia, HR(Croatia), HU (Hungary), RS (Republic of Serbia), BH (Bosnia and Herzegovina), SLO (Slovenia), IT (Italy).

Table 1. List of sampling sites of hard ticks in the study area.

Site no.	Locality	Altitude-latitude (degree/min/sec)	UTM	Vegetation type
1	Seona	45° 28' 24"N, 18° 01' 28"E	BR 74	POHF
2	Viljevo	45° 45' 05"N, 18° 03' 48"E	BR 77	BLF
3	Donji Andrijevci	45° 11' 19"N, 18° 17' 50"E	BR 80	POHF
4	Topoline	45° 33' 41"N, 18° 22' 33"E	BR 84	POHF
5	Koška	45° 32' 44"N, 18° 16' 59"E	BR 84	POHF
6	Sveti Đurađ	45° 44' 24"N, 18° 14' 33"E	BR 86	PF
7	Velika Kopanica	45° 09' 21"N, 18° 23' 40"E	BR 90	POHF
8	Vrpolje	45° 12' 37"N, 18° 24' 19"E	BR 90	POHF
9	Piškorevci	45° 15' 16"N, 18° 23' 57"E	BR 91	POHF
10	Budimci	45° 27' 41"N, 18° 19' 22"E	BR 93	P
11	ustava Krndija	45° 27' 48"N, 18° 23' 09"E	BR 93	POHF
12	Normanci	45° 33' 21"N, 18° 21' 44"E	BR 94	POHF
13	Poganovci	45° 29' 22"N, 18° 23' 59"E	BR 94	POHF
14	Dalj Planina	45° 31' 09"N, 18° 57' 54"E	CR 00	BLF
15	Široko Polje	45° 24' 14"N, 18° 28' 20"E	CR 03	POHF
16	Petrijevci	45° 36' 51"N, 18° 32' 12"E	CR 05	PF
17	Nard	45° 39' 51"N, 18° 28' 49"E	CR 06	PF
18	Haljevo – Beli Manastir	45° 44' 27"N, 18° 36' 42"E	CR 16	POHF
19	Čeminac	45° 41' 12"N, 18° 40' 6"E	CR 16	POHF
20	Popovac	45° 48' 22"N, 18° 39' 34"E	CR 17	BLF
21	Vinkovci	45° 17' 16"N, 18° 48' 20"E	CR 21	POHF
22	Kunjevci	45° 13' 53"N, 18° 48' 04"E	CR 21	POHF
23	Podravlje	45° 33' 53"N, 18° 42' 30"E	CR 24	PF
24	Nemetin	45° 32' 23"N, 18° 46' 15"E	CR 24	PF
25	Bilje	45° 36' 16"N, 18° 44' 39"E	CR 25	PF
26	Lug	45° 39' 48"N, 18° 46' 26"E	CR 25	PODGWF
27	Grabovac	45° 41' 42"N, 18° 44' 34"E	CR 26	PODGWF
28	Mitrovac	45° 42' 34"N, 18° 43' 19"E	CR 26	POHF
29	Branjina	45° 49' 22"N, 18° 41' 35"E	CR 27	BLF
30	Draž	45° 50' 30"N, 18° 47' 18"E	CR 27	BLF
31	Podolje	45° 48' 58"N, 18° 43' 43"E	CR 27	BLF
32	Suza	45° 46' 55"N, 18° 46' 32"E	CR 27	BLF
33	Otok	45° 08' 47"N, 18° 53' 02"E	CR 30	POHF
34	Trpinja	45° 25' 09"N, 18° 53' 57"E	CR 33	P
35	Tikveš	45° 40' 20"N, 18° 50' 37"E	CR 36	PODGWF
36	Zmajevac	45° 48' 03"N, 18° 48' 29"E	CR 37	PF
37	Tompojevci	45° 13' 57"N, 19° 05' 32"E	CR 41	POHF
38	Vukovar	45° 20' 43"N, 19° 00' 04"E	CR 42	BLF
39	Erdut	45° 31' 29"N, 19° 03' 36"E	CR 44	BLF
40	Lovas	45° 13' 34"N, 19° 10' 07"E	CR 50	POHF
41	Sotin	45° 17' 46"N, 19° 05' 48"E	CR 51	BLF
42	Spačva	45° 03' 15"N, 19° 01' 48"E	CQ 38	PODGWF
43	Vrbanja	45° 00' 11"N, 18° 51' 09"E	CQ 38	POHF
44	Budakovac	45° 51' 03"N, 17° 38' 16"E	XL 97	PF
45	Velika	45° 27' 20"N, 17° 39' 45"E	YL 03	POHF
46	Duzluk	45° 30' 55"N, 17° 51' 53"E	YL 24	POHF
47	Orahovica	45° 31' 44"N, 17° 52' 49"E	YL 24	POHF
48	Podravska Moslavina	45° 47' 07"N, 17° 58' 55"E	YL 37	PF

Legend: POHF (Pedunculate oak and hornbeam forests), BLF (Black locust forests), PF (Black and white poplar forests), PODGWF (Pedunculate oak and dyers green weed forests), P (Pasture).

Table 2. Species list, sex ratio, and developmental stages of hard ticks collected in the study area.

Species/Stage	Females	Males	Nymphs	Larvae	Total
Ixodes ricinus	530	375	705	10	1,620
Haemaphysalis inermis	196	118	-	-	314
Dermacentor reticulatus	106	95	-	-	201
Haemaphysalis concinna	9	13	35	-	57
Dermacentor marginatus	12	15	-	-	27
Ixodes canisuga	2	1	-	-	3
Ixodes hexagonus	3	-	-	-	3
Σ	858	617	740	10	2,225

Table 3. Seasonal dynamics of hard ticks in the study area.

Species/Months	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Ixodes ricinus	4♀, 4♂	56♀, 40♂, 153n	197♀, 132♂, 122n	164♀, 141♂, 406n	19♀, 13♂, 2n	15n, 10l	-	7n	10♀, 2♂	29♀, 17♂	51♀, 26♂
Haemaphysalis inermis	17♀,11♂	18♀,16♂	134♀, 86♂	10♀, 4♂	-	-	-	-	-	17♀	1♂
Dermacentor reticulatus	13♀, 4♂	27♀, 4♂	28♀, 10♂	10♀, 5♂	-	-	-	-	2♀	24♀, 69♂	2♀, 3♂
Dermacentor marginatus	2♀, 1♂	2♀, 2♂	4♀, 4♂	2♀, 7♂	-	-	-	-	-	-	2♀, 1♂
Haemaphysalis concinna	-	1♂	2♀, 1♂	5♀, 10♂, 30n	2♀, 1♂	5n	-	-	-	-	-
Ixodes canisuga	-	-	-	2♀, 1♂	-	-	-	-	-	-	-
Ixodes hexagonus	-	-	-	-	-	2♀	1♀	-	-	-	-
Σ	56	319	720	797	37	32	1	7	14	156	86

Legend: ♀ (female), ♂ (male), n (nymph), l (larva).

Table 4. The number of collected hard ticks on domestic animals in the study area.

Species/Host	Cat (*Felis catus*)	Dog (*Canis lupus familiaris*)	Horse (*Equus caballus*)	Sheep (*Ovis aries*)	Σ
Ixodes ricinus	87	139	4	-	230
Dermacentor reticulatus	1	39	-	1	41
Dermacentor marginatus	-	5	-	10	15
Σ	88	183	4	11	286

Table 5. The number of collected hard ticks on wild animals in the study area.

Species/Host	Wild boar (*Sus scrofa*)	Red deer (*Cervus elaphus*)	Hedgehog (*Erinaceus roumanicus*)	Red fox (*Vulpes vulpes*)	Stone marten (*Martes foina*)	European badger (*Meles meles*)	Σ
Ixodes ricinus	-	93	-	3	5	-	101
Dermacentor reticulatus	88	6	-	-	-	-	94
Haemaphysalis inermis	18	-	-	-	-	-	18
Dermacentor marginatus	3	-	-	-	-	-	3
Ixodes hexagonus	-	-	3	-	-	-	3
Ixodes canisuga	-	-	-	-	-	2	2
Σ	109	99	3	3	5	2	221

by the Sørenson index. Differences observed among numbers of ticks collected in different developmental stages (adult, nymph, larva), as well as differences in abundance of tick species on different hosts were tested by Chi-square. P values <0.05 were considered as statistically significant.

RESULTS

A total of 2,225 specimens was collected, belonging to seven species of ticks grouped into the genera *Ixodes*, *Dermacentor*, and *Haemaphysalis* (Table 2). Most ticks were collected by the dragging-flagging method, but 507 ticks were collected on domestic and wild animals (Tables 4 and 5). The most abundant species was *Ixodes ricinus* with 1,620 specimens, comprising 72.8% of the collected ticks, followed by *Haemaphysalis inermis* with 14.1%, *Dermacentor reticulatus* with 9.0%, *Haemaphysalis concinna* with 2.6%, *Dermacentor marginatus* with 1.2%, and *Ixodes canisuga* and *Ixodes hexagonus* representing 0.13% of the collected specimens (Table 2). The largest number (67%) of collected ticks was in the adult stage, while 33.0% were in the nymphal and larval stage (Table 2). In terms of sex ratio, females predominated (Table 2). *Ixodes ricinus* was collected in all developmental stages (Table 2). Other species *D. marginatus*, *D. reticulatus*, *H. inermis*, *I. canisuga*, and *I. hexagonus* were collected only in the adult stage, while *H. concinna* was collected in the nymphal and adult stages (Table 2).

The numbers of collected females, males, and nymphs of *I. ricinus* and *H. concinna* differed significantly ($\chi^2 = 101.57$, P < 0.05; $\chi^2 = 20.62$, P < 0.05). *Ixodes ricinus* nymphs were the most abundant in May, with adults prevalent in April (197 females and 132 males), unlike *H. concinna* that was most abundant in both stages in May (Table 3). Ticks collected on ten different vertebrate hosts were adults (348 females and 159 males). On domestic animals, three tick species were recorded, with six on wild animals (Tables 4 and 5). *Ixodes ricinus* was the most abundant tick species in both groups of animals representing 65.3 % of the collected ticks, followed by *D. reticulatus* with 26.6%, with fewer other species collected (Tables 4 and 5). *Dermacentor reticulatus* was collected in

significantly higher numbers on dogs than on other domestic animals (Table 4). Also, significant differences were found between the numbers of *I. ricinus* collected on cats and dogs ($\chi^2 = 11.96$, P < 0.05). For wild animals, *D. reticulatus* was collected in considerably higher numbers on wild boars than on other animals, while *I. ricinus* was the most abundant on red deer (Table 5). However, the number of collected ticks on wild boars and red deer did not differ significantly ($\chi^2 = 0.48$, P > 0.05). *Ixodes ricinus* and *D. reticulatus* were mainly collected on the head and neck of the domestic animals (cats and dogs). On red deer, the majority of *I. ricinus* was collected on the ventral side of the body in the groin area and the inside of the thighs, while on the wild boars, *D. reticulatus* was mainly collected on the head, ears, and neck. Fourteen domestic animals (one horse, six cats, and seven dogs) were infested with *I. ricinus*. Five dogs and one cat were infested with *D. reticulatus*, while four sheep and one dog were infested with *D. marginatus*. In the group of wild animals, one stone marten, two red foxes, and ten red deer were infested with *I. ricinus*. Furthermore, one European badger was infested with *I. canisuga*, one wild boar with *D. marginatus*, two hedgehogs with *I. hexagonus*, two wild boars with *H. inermis*, and nine wild boars with *D. reticulatus*. Coinfestation with two species of ticks on domestic and wild animals occurred on two dogs and two red deer by *I. ricinus* and *D. reticulatus*, and one sheep was coinfested by *D. marginatus* and *D. reticulatus*, with *D. reticulatus* and *H. inermis* observed on one wild boar. On eighteen inspected wild animals, no ticks were recorded (one wildcat, two red foxes and wild boars, three European hare, hedgehogs, red deer, and four European badgers). Single species infestation with *I. ricinus* was found in 45.8% of animals (domestic and wild), followed by *D. reticulatus* with 25.4%, *D. marginatus* with 10.2%, *H. inermis* and *I. hexagonus* with 3.4%, and *I. canisuga* with 1.7%.

Coinfestation with two species of ticks was recorded in 10.2% of animals. Ten new tick-host associations were recorded for Croatian fauna. *I. ricinus* was hand –picked for the first time from stone marten, red foxes, and red deer, *D. reticulatus* from cats, dogs, sheep, wild boars, red deer, and *D. marginatus* and *H. inermis* from wild boars. Tick samplings

Table 6. List of hard tick species recorded in the study area.

Species/Locality	*I. ricinus*	*H. inermis*	*D. reticulatus*	*H. concinna*	*D. marginatus*	*I.canisuga*	*I. hexagonus*
Seona	+	-	-	+	-	-	-
Viljevo	+	-	-	-	-	-	-
Donji Andrijevci	+	-	+	+	-	-	-
Topoline	+	-	-	-	-	-	-
Koška	+	-	+	+	-	-	-
Sveti Đurađ	+	-	+	-	+	-	-
Velika Kopanica	+	-	+	+	-	-	-
Vrpolje	+	-	-	-	-	-	-
Piškorevci	+	-	-	+	-	-	-
Budimci	-	-	+	-	-	-	-
ustava Krndija	+	-	-	-	-	-	-
Normanci	+	-	+	-	-	-	-
Poganovci	+	-	-	-	-	-	-
Dalj Planina	+	-	-	-	-	-	-
Široko Polje	+	-	-	+	-	-	-
Petrijevci	+	-	-	-	-	-	-
Nard	+	-	-	-	-	-	-
Haljevo – Beli Manastir	+	+	+	+	-	-	-
Čeminac	+	+	-	-	-	-	-
Popovac	+	+	+	-	-	-	-
Vinkovci	+	-	-	-	-	-	-
Kunjevci	+	-	-	-	-	-	-
Podravlje	+	-	+	+	+	-	-
Bilje	+	-	-	-	-	-	-
Nemetin	+	-	-	-	-	-	-
Lug	+	+	+	+	-	-	+
Grabovac	+	+	-	-	-	-	-
Mitrovac	-	-	-	-	-	+	-
Branjina	+	+	-	-	-	-	-
Draž	+	+	+	-	-	-	-
Podolje	+	+	+	-	+	-	-
Suza	-	+	-	-	-	-	-
Otok	+	-	-	-	-	-	-
Trpinja	-	-	+	-	+	-	-
Tikveš	+	+	+	-	+	-	-
Zmajevac	+	+	+	-	+	-	+
Tompojevci	+	-	+	+	-	-	-
Vukovar	+	-	-	-	-	+	-
Erdut	+	-	-	-	-	-	-
Lovas	+	-	-	-	-	-	-
Sotin	+	-	-	-	-	-	-
Spačva	+	-	+	-	-	-	-
Vrbanja	+	-	-	-	-	-	-
Budakovac	+	-	+	-	-	-	-
Velika	+	-	-	+	-	-	-
Duzluk	+	+	-	+	-	-	-
Orahovica	+	-	-	-	-	-	-
Podravska Moslavina	+	-	+	-	-	-	-
Σ 48	44	12	19	12	6	2	2

Table 7. Hard tick abundance in forest communities in the study area.

Species / forest communities	Pedunculate oak and hornbeam forests	Black locust forests	Black and white poplar forests	Pedunculate oak and dyers green weed forests	Σ
Ixodes ricinus	1,018	135	66	171	1,390
Haemaphysalis inermis	141	69	-	104	314
Dermacentor reticulatus	23	18	19	100	160
Haemaphysalis concinna	44	-	12	1	57
Dermacentor marginatus	-	6	3	3	12
Ixodes canisuga	2	1	-	-	3
Ixodes hexagonus	-	-	1	2	3
Σ	1,228	229	101	381	1,939

Table 8. Sørenson index values for tick fauna in four forest communities in the study area.

Study area	Pedunculate oak and hornbeam forests	Black locust forests	Black and white poplar forests	Pedunculate oak and dyers green weed forests
Pedunculate oak and hornbeam forests	-	80.00	60.00	72.72
Black locust forests	-	-	60.00	72.72
Black and white poplar forests	-	-	-	72.72

on animals (domestic and wild) resulted in six tick species (Tables 4 and 5), as well as by the dragging-flagging method. *Ixodes hexagonus* was only collected from run-over wild animals (Table 5), while *H. concinna* only by the dragging-flagging method. The remaining five species (*D. marginatus*, *D. reticulatus*, *H. inermis, I. canisuga* and *I. ricinus*) were collected using both sampling methods. Ticks were recorded during 11 months of the year, except January. During spring months (March, April, May) 82.5 % of ticks were collected (Table 3). *Ixodes ricinus* was recorded in ten months, *D. reticulatus* in seven, while other species were collected in a shorter period (Table 3). *Ixodes ricinus* showed bimodal seasonal activity with the first highest activity peaks recorded in May and the second at the beginning of December (Table 3). Also, bimodal activity patterns were recorded for *D. reticulatus* and *H. inermis* with the first activity peaks in April and the second in November (Table 3). The highest activity peak for *H. concinna* and *Dermacentor marginatus* were noted in May (Table 3). The other two tick species were not analyzed because of the small number of collected specimens. *Ixodes ricinus* was also the most common species in the study area. It was recorded in almost all localities (44/48), followed by *D. reticulatus* in 19 localities, *H. inermis* and *H. concinna* in 12 localities, *D. marginatus* in six localities, *I. canisuga,* and *I. hexagonus* in two localities (Table 6). In pedunculate oak and dyers green weed forests, six tick species were recorded, and in the other three forests communities (black and white poplar, black locust, pedunculate oak and hornbeam) five tick species were recorded (Table 7). In all forest communities *I. ricinus* was the most abundant species (Table 7). Comparison of hard tick faunas in four forest communities via the Sørenson index showed that fauna of black locust forest and pedunculate oak and hornbeam forests were most similar to each other (80.00%), (Table 8). The lowest similarity was found between poplar forests and black locust forests as well as between poplar and pedunculate oak and hornbeam forests (60.00%), (Table 8). Two species, *I. ricinus* and *D. reticulatus,* were collected in all forest communities in the study area (Table 7). Other species (*D. marginatus*, *H. concinna*, *H. inermis*, *I. canisuga,* and *I. hexagonus*) were collected in two or three forest communities (Table 7).

DISCUSSION

The fauna of hard ticks in eastern Croatia has not been sufficiently studied. In the second half of the twentieth century only *D. marginatus* and *D. reticulatus* were recorded in this area (Mikačić 1968, Vesenjak-Hirjan and Šooš 1976). However, during the last six years, ticks were collected in five new localities which resulted in four first-recorded tick species for this area (Krčmar 2012, Krčmar et al. 2014). *Ixodes ricinus* was the most abundant tick species in the studied area. In many field studies or studies of tick infestation of domestic or wild animals conducted throughout Europe, *I. ricinus* was the most abundant species (Milutinović and Radulović 2002, Pavlidou et al. 2008, D' Amico et al. 2017). In the present study, *I. ricinus* was recorded in 91.7% of localities. In a similar study in Hungary, *I. ricinus* was also found frequently (Hornok and Farkas 2009). These findings are not accidental, because the primary habitat of *I. ricinus* is scrubland, deciduous, or

mixed forests that have an abundance of small and large wild vertebrate hosts (Paul et al. 2016). *Ixodes ricinus* does not occuppy open habitats, open hillsides, or homogeneous young coniferous forests (Estrada-Peña 2001). During this study, *I. ricinus* was not recorded in open habitats (pasture) in the villages of Trpinja and Budimci. Also, *I. ricinus* was absent on high altitude mountains (Estrada-Peña et al. 2006), but among all European hard tick species, *I. ricinus* was the most widely distributed and was often recorded as the most abundant species in local tick fauna (Estrada-Peña and Venzal 2006, Sormunen et al. 2016). In this study, *I. ricinus* was represented with 72.8%. which is in agreement with other studies in Europe. For instance, in deciduous woodland in southern England, *I. ricinus* was represented by 96% (Cull et al. 2017), while in green areas of the Belgrade region, *I. ricinus* was predominant with 97.4% in the collected sample (Milutinović et al. 2006).

Ixodes ricinus is considered the most important vector of many zoonotic pathogens of viral and bacterial origin in Europe (Furness and Furness 2018). In Croatia, tick-borne encephalitis virus (TBEV) was isolated from adult *I. ricinus* ticks (Jemeršić et al. 2014), while in neighboring Serbia, tick-borne encephalitis virus (TBEV) was detected in nymphs (Potkonjak et al. 2017). In earlier comprehensive faunistical studies of tick fauna in Croatia, *I. ricinus* was recorded on 24 hosts (Mikačić 1949, 1965, Tovornik 1987, 1990, Tovornik and Brelih 1980). Among these hosts, red deer, red fox, and stone marten were not listed, so the results of this study increased the number of hosts for *I. ricinus* to 27. *I. ricinus* was also recorded for the first time on red foxes in Italy representing new tick host relationships (Lorusso et al. 2011). In Turkey, *I. ricinus* was recorded on 11 hosts (Keskin et al. 2017). A considerably wide host range (63) for this species was recorded in Romania (Coipan et al. 2011, Mihalca et al. 2012). From ticks collected on pet animals (cats and dogs), *I. ricinus* was the most represented. Also, in Great Britain and Hungary, *I. ricinus* occurs in very high densities on pet animals (Ogden et al. 2000, Földvári and Farkas 2005). Prefered sites of attachment of *I. ricinus* on dogs were head and neck rather than other parts of the body. These data correspond with the data obtained in Hungary (Földvári and Farkas 2005). A bimodal activity pattern was described for *I. ricinus* in central Europe (Egyed et al. 2012), while in southern Germany, a unimodal activity pattern occured more often (Schulz et al. 2014). During the summer months, adult females and males of *I. ricinus* were not recorded (Table 3). Also, in southern Italy, during summer, a decline in the number of nymphs and adult ticks of *I. ricinus* was observed (Dantas-Torres and Otranto 2013a). *Haemaphysalis inermis* was the second most abundant tick species in this study. An identical order of species was recorded in forested areas in southern Italy where *I. ricinus* was the most abundant with 69.0%, followed by *H. inermis* with 19.1% (Dantas-Torres and Otranto 2013b). *Haemaphysalis inermis* was collected in 11 localities in eastern parts of the study area (Baranja) and on the western parts in one locality. The average rainfall differs for the eastern and western parts of the study area. In the east it was between 600 and 700 mm and in the west it was between

800 and 900 mm (Vukelić et al. 2008). This may be one reason why *H. inermis* was mostly recorded in the eastern part of the study area, as the optimal annual rainfall for this tick species is between 600 and 833 mm (Perez-Eid et al. 1993).

Haemaphysalis inermis in central Europe had a peak of activity in the autumn (Hornok 2009), while in the study area, the highest activity peak was recorded in the spring. This different seasonality was probably caused by the climatic differences between these biogeographic regions. In the present study, seven times more *D. reticulatus* specimens were collected compared to *D. marginatus*. *Dermacentor reticulatus* was recorded on five hosts, while *D. marginatus* was on three hosts (Tables 4 and 5). Samplings of *D. reticulatus* on cats, dogs, sheep, wild boars, and red deer, as well as *D. marginatus* on wild boars, represent newly recorded tick-host associations in Croatia. According to data from earlier studies, *D. reticulatus* was recorded on two hosts, while *D. marginatus* was on ten hosts (Mikačić 1965, 1968, Tovornik 1988, Tovornik and Matjašić 1991, Krčmar 2012). In Hungary, *D. reticulatus* was the most common tick species on dogs (Földvári and Farkas 2005), but in this study, *D. reticulatus* was recorded as the second most abundant species on dogs. *Dermacentor reticulatus* was collected in the study area in 19 locations, while *D. marginatus* was collected in six (Table 6). This is consistent with the predominance of *D. reticulatus* over *D. marginatus* in western Hungary (Hornok and Farkas 2009). *Dermacentor reticulatus* prefers areas with a moderately moist climate, while *D. marginatus* occurs under a warmer and drier climate (Rubel et al. 2016). *Dermacentor marginatus* was often restricted to the southeastern parts of some countries, under hot and dry climate conditions similar to the Mediterranean type (Estrada-Peña and Santos-Silva 2005). In this study, 37.0% of *D. marginatus* was collected on sheep, its main host (Walter et al. 2016), 18.5% on dogs, 11.1% on wild boars, and 33.3% were collected by the dragging-flagging method in different forest communities. Climatic conditions in the study area belong to the Cfb climate category and may thus better suit *D. reticulatus* than *D. marginatus*. *Dermacentor reticulatus* prefers cooler areas (Rubel et al. 2016), supported by the fact that it is the second most frequently reported tick species after *I. ricinus* in central Europe (Rubel et al. 2016). The seasonal activity of *D. reticulatus* has been extensively studied in Europe and two activity peaks were recorded, in spring and in autumn (Széll et al. 2006, Gračner Jadrešić et al. 2017). Also, in this study, *D. reticulatus* showed a bimodal pattern of activity. The first activity peak was recorded in April and the second in November when the highest activity peak was recorded. A similar activity peak of *D. reticulatus* was recorded in central Europe (Hornok 2009). Monitoring of the distribution of *D. reticulatus* is particulary important from a medical and veterinary point of view because this tick species is a vector of several important pathogens including *Anaplasma marginale*, *Babesia canis*, *B. caballi, Theileria equi*, *Rickettsia raoultii,* tick-borne encephalitis virus, and Omsk haemorrhagic fever virus (Široký et al. 2011, Földvári et al. 2016, Olivieri et al. 2016, Beck et al. 2009). In Croatia, *D. reticulatus* was recognized as having an important role in the transmission of *B. canis* among different domestic or wild

animals (Beck et al. 2017). During the last 20 years, expansion of *D. reticulatus* into new areas had been observed in several European countries, increasing a risk for tick-borne diseases (Kiewra and Czulowska 2013, Földvári et al. 2016, Kubiak et al. 2018). During the 1950s in Hungary, *D. reticulatus* was detected in two isolated areas of two counties, while in the present century it was detected in 16 counties, indicating a high prevalence (Sréter et al. 2005).

In the study area, *D. reticulatus* was recorded for the first time during the 1960s only in one locality (Vesenjak-Hirjan and Šooš 1976), while in this study it was recorded in 19 localities. *Dermacentor reticulatus* is the second most abundant tick species in many parts of Europe after *I. ricinus* (Kubiak et al. 2018). The extensive spread of *D. reticulatus* tick is related to the loss of forest area (Mierzejewska et al. 2017). *Haemaphysalis concinna* was collected in 12 localities by the dragging-flagging method. In central Europe, *H. concinna* was the third most abundant tick species flagged from vegetation (Rubel et al. 2018). A monophasic activity was observed for *H. concinna*. Also, similar activity of *H. concinna* was recorded in central Europe (Hornok 2009). In Hungary, *H. concinna* was active from May to July and disappeared between October and March (Széll et al. 2006), but in this study few adults of *H. concinna* were recorded in March and April (Table 3). *Ixodes hexagonus* was collected from hedgehogs that were run over. This finding represented a second record for the study area. In Great Britain, *I. hexagonus* occurs in a very high density on cats and a significantly smaller density on dogs (Ogden et al. 2000). Only three specimens of *I. hexagonus* were recorded in this study, preventing any comparison with the above-mentioned data. One of the main hosts of *I. hexagonus* is hedgehog (Gern et al. 1991), the likely reason for finding this species on that host. The recent finding of tick-borne encephalitis virus (TBEV) isolated from adult *I. hexagonus* in Croatia (Jemeršić et al. 2014) indicates a high vector potential of this species. Finding *I. canisuga* on the vegetation and on run-over European badgers confirms its recent record from red foxes in Croatia (Hornok et al. 2017).

Thus, there are now 22 ixodid species in the fauna of Croatia. Fifty-three animals were infested with one tick species, while coinfestation with two species of ticks was recorded in six animals. Single species infestation with *I. ricinus* or *D. reticulatus* was found in 27 and 15 animals, respectively. Similar infestation of dogs with these two tick species was observed in Hungary (Földvári and Farkas 2005). In this study, coinfestation with *I. ricinus* and *D. reticulatus* was observed on dogs and red deer. These two tick species often parasitize wild carnivores in Romania (D' Amico et al. 2017). In Italy, coinfestation with two different tick species was found in 1.7% of examined dogs (Maurelli et al. 2018) and in Greece on 6.7% of dogs (Latrofa et al. 2017), while in this study we found coinfestation on 13.3% of dogs. Most of the coinfested animals were parasitized by *D. reticulatus* in association with *D. marginatus*, *H. inermis,* and *I. ricinus*. In Romania, most of the coinfested carnivores were parasitized by *I. ricinus* in association with other tick species (D' Amico et al. 2017). Comparison of tick fauna from four forest communities in the study area showed small differences among them. Comparing presence/absence data with the Sørenson index showed the highest similarity between the tick fauna of black locust forests and pedunculate oak and hornbeam forests. Also, values among other forest communities are very high (Table 8).

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