



Monthly infestation characteristics of ticks on cattle in Thrace, a Crimean Congo hemorrhagic fever-endemic area of Turkey

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Abstract

The first etiologically confirmed cases of Crimean-Congo hemorrhagic fever (CCHF) in humans were detected in Turkey in 2002. Since then, thousands of cases have been reported from different parts of the country. *Hyalomma* (*Hy.*) *marginatum* is considered the main vector tick of CCHF in Turkey, and the primary infection route for humans is known to be the tick bite. This study was carried out between January 2013 and December 2014 in Thrace, Turkey, to determine monthly prevalence and intensity of tick infestation in cattle and, ultimately, to predict the related risk of human exposure to ticks and tick-borne diseases. During the study, 1,701 cattle in 24 villages were screened for ticks; 24,012 adult ticks, 1,887 nymphs, and 766 larvae were encountered on 1,228 of these cattle. On the 1,318 cattle that routinely grazed in the daytime, *Hy. marginatum* was the most predominant species, with an infestation prevalence of 73.6% and an average tick intensity of 16.1 on the infested cattle. In order of prevalence (%) in the grazing cattle, *Hy. marginatum* was followed by *Rhipicephalus* (*R.*) *bursa* (32.7%), *R. turanicus* (29.5%), *Ixodes* (*I.*) *ricinus* (15.1%), *Haemaphysalis* (*Ha.*) *parva* (10.4%), *Ha. inermis* (10%), *Ha. punctata* (6.5%), and *Dermacentor* (*D.*) *marginatus* (2.3%). The obtained data were discussed in terms of the infestation characteristics of the observed tick species, the drivers that may affect these characteristics, and the features of possible relationship between *Hy. marginatum* infestation in the cattle and CCHF cases among humans in the area.

Keywords Cattle · Tick · Season · *Hyalomma* · Thrace · Turkey

Introduction

Ticks are one of the most significant parasites of humans and animals. Many tick species have been well documented to harbor a greater variety of pathogens than any other arthropod groups, including bacteria, viruses, and protozoans (Sonenshine and Roe 2014). Negative influence of ticks

on animals is related to both by tick-transmitted pathogens and their direct damaging effects such as blood loss, irritation, general weakness, and reduction in meat quality and milk production. Cattle ticks are known to be responsible for severe economic losses in both dairy and beef cattle, as the result of yield loss and the cost of infestation combat (Narladkar 2018). Tick infestation in animals has also a special impact on humans. The main two reasons for this significance are the fact that animals can be the source of many zoonotic tick-borne diseases and have a critical role in the maintenance of the vector ticks serving as a host. This relationship is well documented in the natural dynamics of many diseases, such as CCHF. In the Western Palearctic, it has been reported that the epidemiology of CCHF is particularly related to the presence of *Hy. marginatum*, and the abundance of this species is causally associated with the presence of cattle (Ergonul 2012; Estrada-Peña et al. 2013). Infestation prevalence, intensity (tick counts on infested animals), species variety, and seasonal dynamic of the ticks in a region are known to be overly complex and to be affected by many factors, biotic or abiotic, such as availability and

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reachability of the proper hosts, general and seasonal temperature regime, precipitation, moisture, features of habitat, vegetation, and some geographical characteristics. Furthermore, the mode of action and severity of these drivers may also vary dramatically depending on the stages and species of ticks, so this variation is known to be the main reason in the seasonal activation differences between tick species (Needham and Teel 1991; Randolph 2004).

This study was carried out in Thrace, the European part of Turkey. Thrace is geographically located approximately at a central point of the Western Palearctic. The region has a diverse and transitional ecological and weather conditions due to being surrounded by different climatic patterns such as the Black Sea, the Balkans, and the Mediterranean. As a result of these drivers, it has a representative feature of a large part of the Western Palearctic. In addition to all these, high grazing livestock population, rich natural life (Republic of Turkey Ministry of Agriculture and Forestry; <https://www.tarimorman.gov.tr>), and being a bottleneck on an important wild bird migration routes (Kirwan et al. 2014; Najdenski et al. 2018) make Thrace outstanding in terms of some tick species and relevant tick-borne diseases. The study was performed to determine infestation prevalence, infestation intensity, and monthly dynamics of tick infestation in cattle in Thrace. The obtained data was discussed in terms of the infestation characteristics of the tick species, possible drivers that may affect these characteristics, and the features of possible relationship between *Hy. marginatum* infestation in the cattle and CCHF human cases in the area.

Materials and methods

Geographical, climatic, and ecological features of the study area

Thrace, the study area, is bounded by Greece and Bulgaria to the west, the Bosphorus Strait/Istanbul to the east, the Black Sea to the north, the Sea of Marmara to the south, and the Aegean Sea to the southwest. In the field studies, northern part of the region consisting Kirklareli province (41°44'N, 27°13'E, at the center) was especially focused. According to the governmental data (<https://www.tarimorman.gov.tr>, <https://www.saglik.gov.tr>), the CCHF human cases and both total and grazing cattle population are higher in this province than in the other parts of Thrace. In Kirklareli, the human population is around 360,000 (20% in villages, 11% in towns, 69% in urban). Approximate animal inventory in the province during our study period is as follows: more than 142,000 cattle (around 1.5% local breeds, 13.5% crossbred, and 85% European breed as approximately 3/4 Holstein and 1/3 Simmental or other breeds), around 1500 water buffalo, 260,000 sheep, and 62,000 goats. There are around 9,600

livestock farms, and most of them (2/3), especially in the villages, are smallholder (up to 10 cattle) dairy farms. In the villages, most of the farmers use traditional stall housing system, and more than 90% of the cattle graze in temperate and hot months (6–9 months); in a few farms (less than 3%), they graze all year around. Grazing is more prevalent in the villages in relatively mountainous areas.

The surface area of Kirklareli province is 6,650 km² which consisted 48% mountainous terrain on the Black Sea coast (the altitude of the highest point 1,031 m), 35% undulating plateau, and 17% plains. Of the total land, 39% is used for agriculture, 40% is forest (mainly oaks) predominantly at the mountainous area, 5% is pasture, and the remaining 16% is non-agricultural land. There are 3 dams (surface area around 10 km² for each), 34 irrigation ponds, and 165 animal drinking water ponds in the province. Considering Thrace in general, it is seen that large plains and hilly surfaces occupy the interior and south parts of the region. These parts contain predominantly cultivable areas and comprise a belt of bush with short and relatively dry vegetation, maquis in places, and forests in some highlands (mainly oaks). The north side of the region has high mountains which run parallel to the Black Sea, covered by dense, rainy, deciduous forests.

Thrace has a diverse and transitional weather conditions due to being surrounded by different climatic patterns such as the Black Sea, the Balkans, and the Mediterranean. The coasts are affected by the climate of the sea nearby, whereas inland exhibits continental climate to some degree. Summer season is generally hot and moderately rainy, except the Black Sea coast receiving relatively higher precipitation (annual precipitation is up to 900 mm per m²) than the inland (annual precipitation is around 750 mm per m²). The winters are cold and rainy, especially inland experiencing heavy snow and freezing temperatures below 0 °C in places (average annual temperature 13 °C). From January to December, monthly mean temperatures (°C) are 3.0, 4.1, 6.9, 12.1, 17.3, 21.6, 23.9, 23.4, 19.2, 13.9, 9.0, and 5.0 and monthly precipitations (kg/m²) are 61.5, 50.9, 46.7, 44.9, 49.6, 47.7, 24.6, 21.3, 34.1, 53.4, 66.0, and 69.4, respectively (the Turkish State Meteorological Service; <https://www.mgm.gov.tr/>).

Field study and sample collection

The field study covered a period from June 2013 to December 2014 involving 24 Thracian villages (Akpınar, Armağan, Asagikanara, Balkaya, Cakilli, Cayirli, Cesmekoy, Devletliagac, Duzorman, Erikler, Evrenli, Karahamza, Kayali, Kiyikoy, Kizilagac, Kocatarla, Komurkoy, Malkoclar, Omeroba, Suleymandanisment, Tastepe, Vaysal, Kuzulu, and Yoguntas), which are in an area of approximately 3,000 km² (roughly dimensions 25 × 100 km). The visiting periods to the villages and the number of cows

examined are given in Table 1. During the examination, the entire body surfaces of the cattle were screened for the presence of ticks. Larval and nymphal stages (as much as possible) and all the adult ticks were collected in separated vials according to the animals, which were labeled with required information such as ear tag number of the cattle, address, name of the owner, and date. Any special data related to tick infestation, host, barn characteristics, farming procedure and grazing process were recorded in detail. Inspectional data on the attachment sites of the tick species on the animals were recorded. The tick samples were brought to the laboratory and species identification

was performed under stereo microscope using morphological criteria (Estrada-Peña et al. 2017). Fully engorged 269 nymphs and 24 larvae belonging to species *Rhipicephalus bursa* were incubated at 26 °C and 75% relative humidity to moult to make better species identification.

For detecting the possible differences in tick infestation (particularly regarding *Hy. marginatum* and *R. bursa*) among the livestock species, 16 sheep and 45 goats from the four villages (Karahamza, Cayirli, Komurkoy, and Kuzulu), which were grazing in daytime and hold at night in the same farms together with the cattle, were screened for the ticks in 5 months (January, March, April, June, and July) in year 2014.

Table 1 The villages visited during the study, the periods of visits, and the number of cattle examined

Village	Year	Periods of the visits (counts of the cattle examined)		Total counts of the cattle examined
		For wintering cattle	For grazing cattle	
Yoguntas	2014	Jan (24), Feb (13), Mar (3), Nov (6), Dec (9)	–	55
Kocatarla	2014	Jan (26)	–	26
Malkoclar	2014	Jan (10)	–	10
Okcular	2014	Jan (14)	–	14
Tastepe	2014	Jan (16)	–	16
Erikler	2014	Jan (29)	–	29
Kayali	2014	Jan (17)	–	17
Akpınar	2014	Jan (7)	Jan (2)	9
Cesmekoy	2014	Nov (10)	Nov (12)	22
Devletliagac	2014	Jan (49)	July (21)	70
Kiyikoy	2014	Jan (20)	Jan (10)	30
Kizilagac	2014	Jan (4)	Jan (4)	8
Balkaya	2013	–	June (11)	11
Evrenli	2013	–	May (28)	28
Armagan	2013	–	Apr (4), May (9)	13
Cakilli	2013	–	June (14)	27
	2014	–	June (13)	
Asagikanara	2014	–	Jan (15)	15
Omeroba	2014	–	July (17), Nov (23)	40
Suleymandanis	2014	–	July (27)	27
Vaysal	2014	–	July (7)	7
Karahamza	2014	Jan (5)	Jan (1), Mar (15), Apr (16), May (20), June (42), July (27), Aug (21), Sept (18), Oct (14), Nov (26), Dec (21)	226
Duzorman	2013	Dec (30)	Apr (33), May (36), June (62), July (31), Aug (48), Sept (24), Oct (29), Nov (20), Dec (11),	324
	2014	Jan (20), Feb (13), Mar (10)	Apr (30), May (15), June (20)	108
Cayirli	2013	Nov (4), Dec (15)	Apr (47), May (42), June (52), July (27), Aug (51), Sept (18), Oct (27), Nov (14), Dec (9),	306
	2014	Jan (1), Feb (3), Nov (20), Dec (5)	Apr (13), May (12), June (17), July (59), Aug (11), Sept (9), Oct (9), Nov (12)	171
Komurkoy	2013	Apr (18), June (11), July (24), Aug (14)	–	67
	2014	–	Mar (25)	25

Results

During the 2-year study period carried out in the 24 villages of Turkish Thrace (Fig. 1), a total of 1,701 cattle in 94 different family barns containing 1 to 35 cattle were screened for the any presence of ticks. Of those, 1,318 cattle consisted of the animals grazed during the daytime and kept in barns or paddocks at night. The other 383 cattle were kept in the barns for wintering for a period more than 15 days, both night and day. To accurately determine the seasonal dynamic of the tick species in the field under natural circumstance, the data belonging these grazing and wintering cattle groups were evaluated separately.

Of the 383 cattle wintering in the barns constantly in average for 53 ± 20.3 (min–max: 15–90) days, ticks were determined on 42 animals (11.0%) which have been wintering in the barns in average for 44.6 ± 21.6 (min–max: 15–90) days. In these infested cattle, a total of 234 adult ticks (121 ♂, 113 ♀) were encountered and all of them were collected. In addition to these, 22 nymphs and 22 larvae were also collected. The determined tick species (and stages) were as follows: *Rhipicephalus* (*R.*) (*Boophilus*) *annulatus* (larva, nymph, and adult), *R. bursa* (nymph and adult), *Haemaphysalis* (*Ha.*) *parva* (adult), *Ha. punctata* (adult), *Ha. inermis* (adult), *Ixodes* (*I.*) *ricinus* (adult), and *Hy. marginatum* (adult) (Table 2).

Throughout the study, *R. (B.) annulatus* was encountered on the wintering cattle in only two barns in a village

(Yoguntas; 41°56'N, 26°52'E; altitude: 438 m), which were located close to each other (with almost 15 m distance). These barns were visited five times in total in 2014. The first barn was visited in November (on day 15 of wintering) and December. In the first visit, 117 adults, 8 nymphs, and 22 larvae were detected on 3 animals out of 9 cattle in the barn; however, in December, no tick was recorded. The other barn, which was housing 12 cattle, was visited in January (on day 55 of wintering), February, and March. In total, 22 adults and 2 nymphs in January, and 26 adults in February were collected on 2 and 3 cattle, respectively. No tick was recorded in March in this barn. According to our observations, the distinctive feature of these two barns from the others was that they were made of more traditional structure with lower ventilation, higher humidity, and stone walls with many holes and cavities. The walls (inside/outside), roof, paddocks, and surroundings of these two barns were screened to elucidate settlement of engorged female *R. (B.) annulatus* after dropping off the host. In this process, 25 engorged females were found in total, and all the specimens were encountered just in the burrows in the wall at the floor level, and just at parts of the wall where the cattle were tied. It was noticed that most of the ticks which engorged and detached off the host in the winter season began to lay eggs in the burrows in the same winter. In our routine field surveys in the other parts of Thrace outside this study frame, we encountered much more *R. (B.) annulatus* in three different localities: on the cattle hold in a barn in July (Yakupbey village, Meric,

Fig. 1 The villages visited during the field study: 1, Malkoclar; 2, Asagikanara; 3, Devletliagac; 4, Kocatarla; 5, Omeroba; 6, Suleymandeniment; 7, Vaysal; 8, Tastepe; 9, Cesmekoy; 10, Karahamza; 11, Cayirli; 12, Yoguntas; 13, Kayali; 14, Erikler; 15, Kuzulu; 16, Duzorman; 17, Armagan; 18, Akpinar; 19, Komurkoy; 20, Kizilagac; 21, Balkaya; 22, Kiyikoy; 23, Evrenli; and 24, Cakilli



Table 2 Ticks on the cattle wintering in the barns constantly for a determined period

Months	Total counts of cattle examined	Tick species encountered	Counts of cattle infested	Duration of wintering in the barn (day)	Counts of ticks (male–female)
Nov	40	<i>Ha. parva</i>	3	20	4 (2♂, 2♀)
		<i>R. (B.) annulatus</i>	3	15	117 (55♂, 62♀)
		<i>R. (B.) annulatus</i> larva	2	15	22
		<i>R. (B.) annulatus</i> nymph	3	15	8
Dec	59	<i>Ha. inermis</i>	1	30	1 (♀)
		<i>Hy. marginatum</i>	2	25	2 (♂)
			2	60	4 (♂)
		<i>Ha. parva</i>	1	60	2 (♂)
			2	30	3 (♂)
		<i>Ha. punctata</i>	1	30	1 (♂)
		<i>I. ricinus</i>	1	15	1 (♀)
			1	25	1 (♀)
		<i>R. bursa</i>	1	30	1 (♂)
		<i>R. bursa</i> nymph	3	25	10
		<i>Hy. marginatum</i>	4	55	7 (♂)
		<i>Ha. parva</i>	4	55	13 (♂)
			1	25	1 (♂)
		<i>Ha. punctata</i>	2	55	6 (♂)
Jan	242		1	40	1 (♂)
		<i>R. bursa</i>	10	55	17 (9♂, 8♀)
		<i>R. bursa</i> nymph	1	55	2
		<i>R. (B.) annulatus</i>	2	55	22 (6♂, 15♀)
		<i>R. (B.) annulatus</i> nymph	1	55	2
		<i>R. bursa</i>	1	90	1 (♂)
			3	55	3 (2♂, 1♀)
		<i>R. (B.) annulatus</i>	3	90	26 (4♂, 22♀)
		<i>R. bursa</i>	1	90	1 (♂)
Feb	29				
Mar	13				
Total	383		42	Adult	235 (121♂, 113♀)
				Nymph	22
				Larva	22

Edirne Province, 41°14'N, 26°03'E; altitude: 10 m), in a herd grazing on the Black Sea coast (Kiyikoy, Kırklareli Province, 41°38'N, 28°05'E; altitude: 10 m) in June 2012, and on the cattle hold in a barn in July 2016 (Arpac village, Havsa, Edirne Province, 41°41'N, 26°52'E; altitude: 116 m) (no detailed data presented). Apart from these, we did not come across this species in our field research that lasted more than 5 years throughout Thrace.

During the study, the cattle grazing in daytime and kept in the barns or paddocks at night were examined monthly except for February. Of those, 1,318 grazing cattle examined (Table 3) and 1,186 (90.0%) animals were determined as infested by ticks. The monthly prevalence of tick infestation was determined 100% in both March and May, and the least percentage was seen in January as 12.5%. A total of 23,778 adult ticks (63.8% male and 36.2% female), 1,865 nymphs, and 744 larvae were encountered on the animals.

The tick species and their prevalence in grazing cattle were determined as follows: *Hy. marginatum* 73.6%, *R. bursa* 32.7%, *R. turanicus* 29.5%, *R. bursa* nymph 6.8%, *R. bursa* larva 1.8%, *I. ricinus* 15.1%, *Dermacentor (D.) marginatus* 2.3%, *Ha. parva* 10.4%, *Ha. inermis* 10.0%, *Ha. punctata* 6.5%, and *Haemaphysalis* sp. nymph 0.08%. The total counts of the tick species encountered and collected were as follows: *Hy. marginatum* 15,647 (11,183♂-4,464♀), *R. bursa* 2,547 (1,627♂-920♀), *R. turanicus* 3,492 (1,766♂-1,726♀), *R. bursa* nymph 1,840, *R. bursa* larva 744, *I. ricinus* 556 (101♂-455♀), *D. marginatus* 56 (18–38), *Ha. parva* 715 (323♂-392♀), *Ha. inermis* 567 (66♂-501♀), *Ha. punctata* 198 (79♂-119♀), and *Haemaphysalis* sp. nymph 25. We would like to point out that although we collected as much of them as possible, the counts of the nymphs and especially larvae possibly show less than what were present on the animals. The related counts were given to indicate possible

Table 3 Monthly prevalence, intensity, and counts of the tick species in grazing cattle

a		Jan	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Months													
Cattle counts examined		32	40	161	162	242	240	145	69	79	107	41	1,318
Tick counts		8	962	4,152	8,386	5,916	2,237	431	56	301	3,416	522	26,387
Infested cattle counts		4	40	157	162	240	237	117	32	54	103	40	1,186
Total tick prevalence (%)		12.5	100	97.5	100	99.2	98.8	80.7	46.4	68.4	96.3	97.6	90.0
Adult tick intensity (standard deviation) (min–max)		2	24.0	26.5	51.8	24.7	9.5	3.7	1.8	4.8	12.1	5.3	20.3
<i>H. marginatum</i>		(± 1.2)	(± 18.7)	(± 28.4)	(± 36.7)	(± 20.5)	(± 8.8)	(± 2.7)	(± 1.2)	(± 5.0)	(± 10.2)	(± 6.2)	(± 25.4)
		(1–3)	(1–59)	(1–161)	(2–185)	(1–118)	(1–51)	(1–14)	(1–6)	(1–24)	(1–60)	(1–31)	(1–185)
Tick counts		2	624	2,655	6,453	3,920	1,518	361	42	42	27	3	15,647
Prevalence (%)		3.1	75.0	90.7	99.4	95.9	92.5	71.0	37.7	32.9	18.7	7.3	73.6
Intensity (standard deviation) (min–max)		2.0	20.8	18.2	40.1	16.9	6.8	3.5	1.6	1.6	1.4	1.0	16.1
		(± 0.0)	(± 16.4)	(± 20.4)	(± 26.5)	(± 16.1)	(± 5.8)	(± 2.4)	(± 0.8)	(± 1.4)	(± 0.8)	(± 0.0)	(± 20.2)
		(2–2)	(1–52)	(1–155)	(1–116)	(1–112)	(1–38)	(1–12)	(1–3)	(1–8)	(1–4)	(1–1)	(1–155)
Tick counts		–	39	8	2	–	–	–	–	2	5	–	56
Prevalence (%)		–	37.5	4.4	1.2	–	–	–	–	2.5	3.7	–	2.3
Intensity (standard deviation) (min–max)		–	2.6	1.1	1.0	–	–	–	–	1.0	1.3	–	1.9
			(± 2.0)	(± 0.4)	(± 0.0)					(± 0.0)	(± 0.5)		(± 1.6)
			(1–7)	(1–2)	(1–1)					(1–1)	(1–2)		(1–7)
Tick counts		3	186	91	47	34	1	1	–	26	163	4	556
Prevalence (%)		3.1	80.0	19.3	19.8	7.9	0.4	0.7	–	22.8	56.1	9.8	15.1
Intensity (standard deviation) (min–max)		3	5.8	2.9	1.5	1.8	1.0	1.0	–	1.5	2.7	1.0	2.8
		(± 0.0)	(± 4.7)	(± 3.9)	(± 1.5)	(± 1.2)	(± 0.0)	(± 0.0)		(± 0.8)	(± 2.1)	(± 0.0)	(± 3.1)
		(3–3)	(1–15)	(1–19)	(1–9)	(1–5)	(1–1)	(1–1)		(1–4)	(1–9)	(1–1)	(1–19)
b													
<i>R. turanicus</i>													
Tick counts		–	15	1,384	1,762	254	67	8	2	–	–	–	3,492
Prevalence (%)		–	22.5	64.0	83.3	34.7	20.8	4.8	1.5	–	–	–	29.5
Intensity (standard deviation) (min–max)		–	1.7	13.4	13.1	3.0	1.3	1.1	2	–	–	–	9.0
			(± 0.7)	(± 22.6)	(± 18.5)	(± 3.4)	(± 0.6)	(± 0.4)	(± 0.0)				(± 16.9)
			(1–3)	(1–89)	(1–97)	(1–19)	(1–3)	(1–2)	(2–2)				(1–97)
Tick counts		–	–	1	120	1,708	651	61	6	–	–	–	2,547
Prevalence (%)		–	–	0.6	25.9	81.4	60.4	29.0	5.8	–	–	–	32.7
Intensity (standard deviation) (min–max)		–	–	1.0	2.9	8.8	4.5	1.5	1.5	–	–	–	5.9
				(± 0.0)	(± 1.9)	(± 10.2)	(± 5.0)	(± 0.9)	(± 1.0)				(± 7.9)
				(1–1)	(1–9)	(1–61)	(1–24)	(1–4)	(1–3)				(1–61)
<i>Rhipi. larva</i>													
Tick counts		–	–	–	–	–	–	–	–	3	708	33	744
Prevalence (%)		–	–	–	–	–	–	–	–	2.5	15.0	12.2	1.8
Intensity (standard deviation) (min–max)		–	–	–	–	–	–	–	–	1.5	42.3	6.6	32.4
										(± 0.7)	(± 27.7)	(± 2.3)	(± 29.4)
										(1–2)	(1–80)	(3–9)	(1–80)
<i>Rhipi. nymph</i>													
Tick counts		–	1	–	–	–	–	–	–	46	1473	320	1,840
Prevalence (%)		–	2.5	–	–	–	–	–	–	16.5	45.8	65.9	6.8
Intensity (standard deviation) (min–max)		–	1	–	–	–	–	–	–	3.5	30.1	11.9	20.4
			(1–1)							(± 4.9)	(± 32.0)	(± 13.9)	(± 27.1)
										(1–18)	(1–90)	(1–55)	(1–90)

Table 3 (continued)

<i>Ha. inermis</i>	Tick counts	3	44	7	2	—	—	—	—	—	3	458	50	567
	Prevalence (%)	5.8	42.5	3.7	1.2	—	—	—	—	—	3.8	76.6	46.3	10.0
	Intensity (standard deviation) (min–max)	1.0 (±0.0) (1–1)	2.6 (±2.0) (1–7)	1.2 (±0.4) (1–2)	1.0 (±0.0) (1–1)	—	—	—	—	—	1.0 (±0.0) (1–1)	5.6 (±4.8) (1–25)	2.6 (±3.1) (1–14)	4.3 (±4.4) (1–25)
<i>Ha. parva</i>	Tick counts	—	31	—	—	—	—	—	—	6	156	440	82	715
	Prevalence (%)	—	20.0	—	—	—	—	—	—	7.3	38.0	70.1	46.3	10.4
	Intensity (standard deviation) (min–max)	—	3.9 (±4.8) (1–13)	—	—	—	—	—	—	1.2 (±0.5) (1–2)	5.2 (±5.1) (1–21)	5.9 (±6.2) (1–34)	4.3 (±3.9) (1–16)	5.2 (±5.5) (1–34)
<i>Ha. punctata</i>	Tick counts	—	22	6	—	—	—	—	—	—	23	117	30	198
	Prevalence (%)	—	25.0	3.7	—	—	—	—	—	—	16.5	40.2	31.7	6.5
	Intensity (standard deviation) (min–max)	—	2.2 (±1.4) (1–4)	1.0 (±0.0) (1–1)	—	—	—	—	—	—	1.8 (±1.2) (1–5)	2.7 (±2.4) (1–13)	2.3 (±2.1) (1–8)	2.3 (±2.1) (1–13)
<i>Ha. nymph</i>	Tick counts	—	—	—	—	—	—	—	—	—	—	25	—	25
	Prevalence (%)	—	—	—	—	—	—	—	—	—	—	1.3	—	0.08
	Intensity (standard deviation) (min–max)	—	—	—	—	—	—	—	—	—	—	25 (25–25)	—	25 (25–25)

burden and possible seasonal distribution patterns of these stages.

Among the species detected in the study, *Hy. marginatum* (15,647 adults; 59.3% of total ticks, 65.8% of total adult ticks) was the species which had highest prevalence (mean annual prevalence 73.6%) and mean intensity (16.1) level in grazing cattle, and longest activation period during the year with the peak in May (prevalence 99.4%, intensity 40.1 ± 26.5). Of the *Hy. marginatum* adults collected, 71.5% were males, and from March to January, the monthly distribution of the male rate (counts of ♂-♀) was as follows: 84.1% (525–99), 76.7% (2,036–619), 70.5% (4,546–1,907), 68.0% (2,667–1,253), 70.1% (1,064–454), 69.3% (250–111), 73.8% (31–11), 85.7% (36–6), 85.2% (23–4), 100% (3–0), and 100% (2–0), respectively.

According to the qualitative/inspectional data on the attachment sites of the tick species on the cattle, the top three body parts preferred by the species were as follows: inguinal (udder/groin), perineal, and ventral abdominal regions for *Hy. marginatum*; perineal, inguinal, and perianal regions for *R. bursa*; inguinal, ventral abdominal, and perineal regions for *R. turanicus*; tip of tail, withers, and poll area for *D. marginatus*; ventral abdominal region, axillar region, and ventral part of neck for *Haemaphysalis* spp.; inguinal region (inner face of flank and intermammary groove), axillar region, and ventral abdominal region for *I. ricinus*; ventral part of neck (dewlap), axillar region, and ventral abdominal region for *B. (R.) annulatus*; ear, poll, and head for the larvae of *R. bursa*; neck, perianal region, and perineal region for the nymphs of *R. bursa*; and shoulder (withers, crops, and scapular area), posterior part of neck, and dorsal part of thoracal region for the nymphs of *Hameaphysalis* spp.

In the study conducted on grazing sheep and goats, the counts of animals and the tick species determined by months were as follows: in January, four *R. bursa* nymphs and two (♀) *Ha. inermis* on ten goats; in March, 79 (53♂, 26♀) *Dermacentor marginatus*, ten (8♂, 2♀) *Ha. inermis*, and one *Ixodes* sp. nymph on 11 goats; in April, 75 larvae and 206 nymphs of *R. bursa*, one (♀) *D. marginatus*, and six (3♂, 3♀) *R. turanicus* on ten goats; in June, 69 (47♂, 22♀) *R. bursa* and two (♂) *Hy. marginatum* (found on two animals) on 12 goats, and 26 (15♂, 11♀) *R. bursa* and six (♂) *Hy. marginatum* (found on four animals as one or two specimens) on ten sheep; in July, three (two ♂, one ♀) *R. bursa* on four sheep, and no ticks was encountered on two goats examined in this month.

Discussion

In this study, nine different tick species belonging to the five genera (*Hyalomma*, *Rhipicephalus*, *Haemaphysalis*, *Ixodes*, and *Dermacentor*) were determined on the cattle in

Thrace. The general characteristics of their seasonal dynamics were basically concurrent with the previous data reported from similar climate zones of the Western Palearctic such as Aegean coast of Anatolia (Bakirci et al. 2012), Bulgaria (Arnaudov and Arnaudov 2017), Greece (Papadopoulos et al. 1996), and Spain (Castellà et al. 2002). However, our study differs from most of these in terms of the multiple tick infestation, in which each species participated with a significant level of prevalence and intensity (Table 3). Considering the relatively small study area and the bioecological differences between the species (Uspensky 2002; Estrada-Peña et al. 2017), our results appear to be assertive in some respects. However, there are some possible factors that may have been effective at this point. For example, Thrace contains many different climatic and geographic foci in its relatively small area, and the region has a rich wildlife and grazing livestock population (<https://www.tarimorman.gov.tr>). It is well known that some tick species such as *I. ricinus* can survive and maintain its activity in restricted microhabitats with favorable conditions within the regions where general prevailing circumstances are unfavorable (Randolph 2004), and this also applies to most of the other tick species, at least under some special circumstances such as the presence of reachable hosts at enough number (Sonenshine and Roe 2014).

The level of tick intensity in the animals basically is associated with tick population density in the area and the probability of the encountering of the ticks and animals (Randolph 2004; Sonenshine and Roe 2014). However, individual differences in total tick burden among the animals in same herds or farms are frequently encountered in this study. Some factors have been cited as the possible reasons for this variation, such as features of coat, habits, and opportunity of grooming (Shyma et al. 2015), age, lactation, skin thickness, host size (Robbertse et al. 2017), breed of the cattle (Shyma et al. 2015), and sex as related to impaired immunological response to the ticks because of the testosterone (Randolph 2004). Our main study population consisted of female grazing cattle older than 6 months, which were owned by mostly traditional family farms. According to information from the farmers, the number of annually added new animals to the herds was less than 1%, and animal trade is mostly carried out between closer villages. Furthermore, animal transport from Anatolian part of Turkey to Thrace is prohibited by law. Considering this fact, most of the animals in the study area have been most probably exposed to the high tick prevalence and intensity for years. Acquired resistance based on humoral and cellular immunity to feeding ticks was reported to act in a density-dependent manner (Randolph 2004). However, despite the long-term severe infestation, the high tick burden seen in our study area may indicate that the acquired immunity can be helpful in reducing the intensity to a determined level (Wikel and Whelen 1986), but this

may not also mean dramatic diminishing of tick counts in general cattle population. At this point, breeds of the cattle population may be considered another possible driver on the tick intensity. It is well known that strength and degree of acquired immunity against ticks varies significantly among cattle breeds, and *Bos taurus indicus* (Zebu cattle) has obvious advantage over *B. t. taurus* (Robbertse et al. 2017). Furthermore, innate resistance against ticks, which is known to be heritable, may also dramatically change between cattle breeds, even among the *B. t. taurus* (Shyma et al. 2015); e.g., Australian Belmont Red (Burns et al. 1997) and South African Bonsmara cattle (Rechav and Kostrzewski 1991; Fourie and Kok 1995) are reported to be more resistant to some European breeds, e.g., Holstein and Simmental. All these data showed that some European cattle breed have relatively weak resistance to the tick infestation, at least in the environment where they are not indigenous. Under this circumstance, it seems that genetic resistance may be one of the possible reasons for high tick intensity in the cattle in Thrace which consists mostly non-indigenous cattle breed such as Holstein, Simmental, Brown Swiss, and their cross bred.

It is known that some *Hyalomma* species, which prefer cattle as the host, such as *Hy. marginatum*, *Hy. excavatum*, and *Hy. anatolicum*, and two host biological form of *Hy. scupense* (syn. *Hy. detritum*) can inhabit in regions which have similar environmental and climatic conditions (Gray et al. 2009; Estrada-Peña et al. 2017). However, we have detected just *Hy. marginatum* and one host biological form of *Hy. scupense* (unpublished data), which is a typical winter tick (Estrada-Peña et al. 2017), as the resident population in the cattle. Thrace is a bottleneck on the Black Sea Flyway which is one of the most important wild bird migration routes in the world used by millions of birds containing many ground-feeding species. This route extends between South Africa, Madagascar, especially eastern part of Sub-Saharan Africa, Rift Valley, the Nile Delta, and Mediterranean coast of Middle East and Anatolia, Thrace, Europe, and north side of Black Sea (Kirwan et al. 2014; Najdenski et al. 2018). The birds using this route has been reported to carry many kinds of tick species in both directions between long distances, such as immature stages of *Hy. marginatum*, *Hy. rufipes*, and *Hy. aegyptium* (Hoogstraal et al. 1961; 1963). During our field studies other than this research, we have rarely encountered only a few specimens of *Hy. rufipes* on the cattle in different parts of Thrace (data not shown), and on the horses in just south of Thrace, in Anatolia (Kar et al. 2009). Although there is no detailed data related to other species, it is understood that *Hy. rufipes* cannot establish in Thrace as it is true for some other parts of Europe, possibly as the result of cold winter season (Hasle 2013). At this point, it may be taken into consideration that ticks carried by birds coming from different parts of Europe and north of the

Black Sea in autumn may be more successful to establish in the region due to some similarities of climatic, ecological, and geographical conditions. As for *Hy. aegyptium*, presence of this species on tortoises is well known in Thrace with high prevalence and intensity (Kar et al. 2020), and it seems possible to associate the absence of this species on the cattle with its high host specificity. On the other hand, considering the geographical location, climatic condition, and cattle population of Thrace, complete lack of ticks like *Hy. excavatum*, *Hy. anatolicum*, and two-host type of *Hy. scupense*, which are often infesting cattle in Anatolia, needs to be further investigated.

In this study, *Hy. marginatum* was the species which had the highest prevalence and intensity level in grazing cattle, and which is encountered on the animals for the longest time throughout the year (mean annual prevalence 73.32%, intensity 14.93, percentage in total tick count 57.5%, percentage in adult tick counts 63.9%) (Table 3). The adults of this species were seen almost throughout the year with different prevalence and intensity, peaked in May (prevalence 97.0%, intensity 39.9) and June (prevalence 94.7%, intensity 16.8). Although the prevalence and intensity were lower, similar yearlong activation period has been reported from Bulgaria (intensity 1–3, prevalence 28.1%) (Arnaudov and Arnaudov 2017), Greece (intensity 0–47) (Papadopoulos et al. 1996), some Mediterranean parts of Europe (intensity 2.4–13.7, percentage in total tick count 5.3–21.5%) (Torina et al. 2006; Grech-Angelini et al. 2016), and the Aegean coast of the Western Anatolia (44.4% in May as the peak, percentage in total tick count 33.5%) (Bakirci et al. 2012). The studies (Mamak et al. 2006; Tonbak et al. 2006; Gunes et al. 2011; Aktas et al. 2012) carried out in Kelkit Valley and its extensions (Fig. 1), which is CCHF hyperendemic area of Turkey, have revealed shorter activation period mostly limited in temperate months. This is probably because the area has more continental and harsher climate than Thrace (<https://www.mgm.gov.tr/>). The data related to tick infestation characteristics in Thrace and Kelkit Valley and the CCHF cases reported from these two regions do not seem compatible with each other. In Turkey, 11,041 human cases with 4.8% fatality rate have been recorded from 2002 to end of 2018, and most of the cases (around 95%) have been reported from Kelkit Valley and its extensions (<https://www.saglik.gov.tr>). The case counts decrease from this hot spot to the west and south of Anatolia. For example, although CCHFV was detected in 51.5% of unfed questing *Hy. marginatum* adults in Thrace (Akyildiz et al. 2020) and human seropositivity was determined as 25% in Cayirli, 15% in Komurkoy villages (Midilli et al. 2009; Gargili et al. 2011), and different tick species are known to bite human beings in Thrace (Gargili et al. 2010), the number of the CCHF cases recorded between 2005 and 2018 are only 41 in Thrace (23 in Kırklareli). The average annual prevalence of the cases in

Thrace (Kırklareli province) is around 2.9, and it is almost 30 times less than Kelkit Valley (Tokat province, hottest spot of Kelkit Valley for CCHF) when only the density of the population at the risk in two areas is considered.

Conclusion

Our study showed that the tick infestation in the Thrace cattle has a special position in some respects such as species diversity, prevalence, intensity, and the length of active season, compared to most of the other parts of Anatolia and Europe. This is most likely the result of the diverse and rich biotic and abiotic factors of the region, which are described in detail in the “Materials and methods” section. Our results related to *Hy. marginatum* showed that the population of this tick species is stronger in many respects in Thrace than in Kelkit Valley, CCHF hyperendemic area in Anatolia. This indicates that the number of CCHF cases and the population of the vector in a region may not always be causally related. Apparently, it seems that there must be more complex factors affecting the natural dynamics of CCHF, and Thrace may be one of the ideal areas to reveal some of those possible drivers.

Author contribution The design of the experiment was made by Z. V. and S. K. S. K. and G. A. contributed to the field experiment. Tick identification was carried out by S. K. S. K., D. B., and E. G. contributed to the planning, evaluation of the results, and writing of the final manuscript. All authors read and approved the final manuscript.

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