Species Diversity of Ixodid Ticks Feeding on Humans in Amasya, Turkey: Seasonal Abundance and Presence of Crimean-Congo Hemorrhagic Fever Virus

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ABSTRACT Ticks (Acari: Ixodidae) are important pests transmitting tick-borne diseases such as Crimean-Congo hemorrhagic fever (CCHF) to humans. Between 2002 and 2009, numerous CCHF cases were reported in Turkey, including Amasya province. In the current study, species diversity, seasonal abundance of ticks, and presence of CCHF virus (CCHFV) in ticks infesting humans in several districts of Amasya province were determined. In the survey, a total of 2,528 ixodid ticks were collected from humans with tick bite from April to November 2008 and identified to species. *Hyalomma marginatum* (18.6%), *Rhipicephalus bursa* (10.3%), *Rhipicephalus sanguineus* (5.7%), *Rhipicephalus (Boophilus) annulatus* (2.2%), *Dermacentor marginatus* (2.5%), *Haemaphysalis parva* (3.6%), and *Ixodes ricinus* (1.6%) were the most prevalent species among 26 ixodid tick species infesting humans in Amasya province. *Hyalomma franchinii* Tonelli & Rondelli, 1932, was a new record for the tick fauna of Turkey. The most abundant species were the members of *Hyalomma* and *Rhipicephalus* through summer and declined in fall, whereas relative abundances of *Ixodes* and *Dermacentor* ticks were always low on humans in the province. Of 25 *Hyalomma* tick pools tested, seven pools were CCHFV positive by reverse transcription-polymerase chain reaction. Results indicated diversity of ixodid tick species infesting humans was very high, abundance of ticks changed by season, and ticks infesting humans had potential for transmitting CCHFV.

KEY WORDS Amasya, ticks, CCHFV, human, Turkey

In the world, there are 683 ixodid ticks species from 13 genera (*Ixodes, Amblyomma, Anomalohimalaya, Bothriocroton, Cornupalpatum, Cosmiomma, Dermacentor, Haemaphysalis, Hyalomma, Margaropus, Nosomma, Rhipicentor,* and *Rhipicephalus*) in the family Ixodidae (Horak et al. 2002a, Barker and Murrel 2004, Guglielmone et al. 2009). In contrast, ixodid tick fauna of Turkey appear to consist of only 39 species from six genera (Kurtpinar 1954, Hoogstraal 1959, Merdivenci 1969, Ozkan 1978, Erman et al. 2007, Bursali et al. 2008, 2009, 2010) because of very limited number of systematic studies on ticks.

Ixodid ticks play an important role for the transmission of numerous pathogens threaten human health, such as Crimean-Congo hemorrhagic fever (CCHF) virus (CCHFV) (Horak et al. 2002b, Whitehouse 2004), which caused numerous fatal cases between 2002 and 2008 in Turkey, including Amasya province (Ergonul 2006, Com 2008, Yilmaz et al. 2009, The Republic of Turkey Ministry of Health 2010a). The first CCHFV case was reported from Tokat, a neighbor of Amasya province of Turkey in 2002 (Ergonul 2006). Since then, both number of tick bite cases and tick bite-associated CCHFV cases increased in several provinces of Turkey. Between 2002 and 2008, tick bite cases were increased from several to ~7,000 bites per year in Tokat province and >2,000 in Amasya province, indicating an increase in tick numbers in these provinces (Bursali et al. 2009, this study). Twenty of 242 cases of CCHFV were confirmed in Tokat, Sivas, Gumushane, Amasya, Yozgat, and Corum provinces in 2006 (CIDRAP 2006), and most of them were associated with tick bites (Gozalan et al. 2007). In 2008, there were 1,308 confirmed CCHF cases with 135 deaths reported in Turkey, including 46 cases with two deaths from Amasya province (Com 2008, Amasya Department of Health). By September 2009, 62 deaths were reported among 1,300 CCHF cases in Turkey (The Republic of Turkey Ministry of Health 2010b). In general, both tick bites and CCHFV cases reported in Turkey were more prevalent on (although not limited to) people living in rural areas (Ergonul 2006; Bursali et al. 2009, 2010; Tekin et al. 2010).

The studies on ticks infested on humans in South Africa (Horak et al. 2002b), South America (Guglielmone et al. 2006), Argentina (Nava et al. 2006), and Turkey (Vatansever et al. 2008; Bursali et al. 2009,

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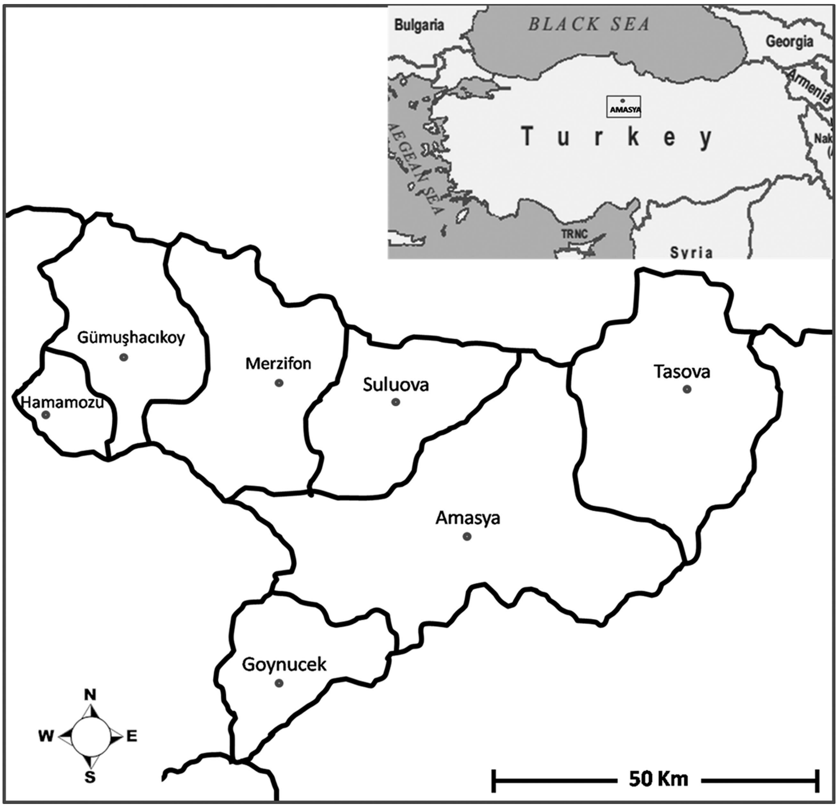

Fig. 1. Map of Turkey showing geographical location and districts of Amasya province.

2010) showed that a variety of tick species infest on humans and they might act as a potential vector of tick-transmitted diseases. *Hyalomma* and *Rhipicephalus* species were more prevalent than others among ticks collected from humans in Turkey (Vatansever et al. 2008; Bursali et al. 2009, 2010). Presence of CCHFV has been detected in numerous ixodid tick species, including ticks infesting on humans in our recent molecular survey (Tekin et al. 2009), indicating that they play a role in transmission of tick-borne diseases.

Even though we recorded >2,000 tick bite cases (this study) and 46 confirmed CCHF cases with two deaths in Amasya province in 2008 (Com 2008, Amasya Department of Health), there is very limited information about species diversity, seasonal abundance, and CCHFV prevalence of ixodid ticks infesting humans in Amasya. In the current study, we performed a survey (from April to November 2008) to determine the species diversity, seasonal abundance, and CCHFV prevalence of ixodid ticks infesting humans in Amasya province of Turkey.

Materials and Methods

Characteristics of Study Area. Amasya province is located in between Central Black Sea and Central Anatolia regions with geographical coordinates of 41°04′54″– 40°16′16″ North and 34°57′06″–36°31′53 East (Fig. 1). Amasya has an area of 5,690 km^2, altitudes range from 190 to 2,062 m, and there is a semiarid to cold Mediterranean climate (Kinalioglu 2009). The mean annual precipitation is 430.8 mm, and the rainfall regime is East Mediterranean Rain Regime type I. The mean annual maximum temperature is 30.4°C in August, whereas the mean minimum temperature is ≈1°C in January (Kinalioglu 2009). Cattle, sheep, and goats are the main domestic stocks of the province. Wild animals such as wild boars, small rodents such as hares, reptiles and ground-feeding birds such as partridges, which are major hosts for ticks, are abundant in the fauna of the province. Information about the prevalence and species diversity of ticks on these animals, however, is very limited.

Collection and Identification of Ixodid Ticks. The current study was conducted using ticks from six districts (Amasya City [the Central District], Goynucek, Gumushacikoy, Merzifon, Suluova, Tasova) of Amasya province. Total of 2,528 ixodid ticks (1,925 adults, 593 nymphs, and 10 larvae) attached to human skin was collected from 2,464 tick-infested humans visiting health centers by health personnel under aseptic conditions, stored in 70% ethanol, and deposited to our acarology laboratory for identification. Of 2,528 ticks, 1,925 adult ticks were identified to species based on the keys given by Nuttall and Warburton (1911, 1915), Kratz (1940), Feldman-Muhsam (1954), Kurtpinar (1954), Hoogstraal (1959), Parrish (1961), Kaiser and Hoogstraal (1964), Nemenz

(1967), Merdivenci (1969), Ozkan (1978), and Walker et al. (2000), and recent world tick lists reported by Horak et al. (2002a), Barker and Murrel (2004), and Guglielmone et al. (2009). The larvae and nymphs were not examined to avoid misidentification because of damaged or missing body parts. Total of 250 *Hyalomma* ticks identified to species was stored at −70°C for testing presence of CCHFV.

Viral RNA Isolation and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). To test presence of CCHFV in ticks, randomly selected *Hyalomma* ticks, which are the most common vectors of CCHFV in Turkey, were pooled to make 25 tick pools of 10 ticks without grouping ticks in species and stored in −70°C. Viral RNA was isolated using High Pure Viral RNA isolation kit (Roche Diagnostics, Mannheim, Germany), according to manufacturer's protocol. Ticks were placed in sterile petri dish and cut half in the middle, and internal organs were removed by scraping with a scalpel blade and crushed in a 2 ml RNase/DNase-free microcentrifuge tube containing 500 μl of homogenization buffer from High Pure Viral RNA isolation kit (Roche Diagnostics), using a sterile plastic pestle. The homogenate was centrifuged at 2,000 × *g* for 5 min, and 250 μl of supernatant was used for viral RNA isolation. The viral RNA was eluted in 40 ml of RNase-free water and stored at −86°C.

Presence of CCHFV in tick pools was tested by one-step RT-PCR using Transcriptor One Step RT-PCR kit (Roche Diagnostics, Mannheim, Germany) according to manufacturer's protocol, using 2–4 μl of RNA. Briefly, in all RT-PCR tests, reaction volume was 25 μl, and a RT-PCR product of 536 bp was amplified using 0.2 μM CCHFV S segment-specific forward (5′-TGGACACCTTCACAAACTC-3′) and 0.2 μM reverse (5′-GACAAATTCCCTGCACCA-3′) primers. Reaction conditions were 55°C for 30 min, 94°C for 10 min, 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 10 s and 72°C for 5 min for final elongation. In all assays, RNA (GU324490) from a CCHFV-positive tick was used as a positive control, and RNase-free water was used as a negative control. The presence of CCHF virus in *Hyalomma* tick pools was confirmed by visualizing the 536-bp part of CCHFV nucleoprotein gene amplified with RT-PCR on a 2% agarose gel (Fig. 2).

Phylogenetic Analysis of CCHFV. For the phylogenetic analysis of CCHFV, PCR products from four different *Hyalomma* tick pools obtained by RT-PCR were purified and sequenced at RefGen (Gen Araçstırmaları ve, Biyoteknoloji, Ankara) using an ABI 3130XL Genetic Anaylzer (Applied Biosystems, Foster City, CA) with a BigDye Cycle Sequencing kit (Applied Biosystems). The CCHFV nucleoprotein partial sequences obtained in the current study (GenBank accession numbers: GU550068, GU550069, GU550070, and GU550071) were used to generate a phylogenetic tree along with similar sequences obtained from National Center for Biotechnology Information GenBank through BLAST search (Altschul et al. 1990). Among >250 CCHFV nucleoprotein sequences returned after conducting a BLAST search, nucleoprotein gene sequences originated from different parts of the world, in addition to Turkey's neighbors, were chosen to construct a phylogenetic tree. BioEdit 7.0.4.1 (Hall 1999) and MEGA4 (Tamura et al. 2007) were used to analyze the sequences and to construct the phylogenetic tree (Fig. 3).

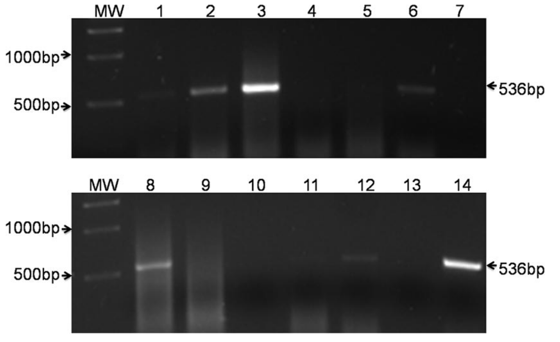

Fig. 2. Detection of CCHFV presence in *Hyalomma* tick pools by RT-PCR. Results of two independent RT-PCR assays are displayed. MW, molecular weight standard; lanes 1, 2, 3, 8, 11, and 12, CCHFV-positive tick pools; lanes 4, 5, and 10, CCHFV-negative tick pools; lanes 6 and 14, positive controls; lanes 7 and 13, negative controls.

Results

Species Diversity and Relative Abundance of Ixodid Ticks Infesting Humans in Amasya Province. Ixodid tick species infesting humans in Amasya were determined using 1,925 adult tick samples only. As shown in Table 1, 26 ixodid tick species were found on humans in Amasya. *Hyalomma marginatum, Hyalomma detritum, Hyalomma turanicum, Hyalomma aegyptium, Rhipicephalus bursa, Rhipicephalus turanicus, Rhipicephalus sanguineus, Haemaphysalis parva, Dermacentor marginatus*, and *Ixodes ricinus* were the most prevalent species (Table 1). Of 26 ixodid ticks, *Ixodes redikorzevi, I. ricinus, Ixodes hexagonus, Dermacentor daghestanicus, Hyalomma dromedarii, Hyalomma isaaci, Hyalomma rufipes, H. turanicum, Haemaphysalis erinacei*, and *Rhipicephalus turanicus* were new records for Amasya province, and *Hyalomma franchinii* was a new record for the ixodid tick fauna of Turkey, according to ixodid tick list from Parrish (1961) and Merdivenci (1969) (Table 1).

In the current study, of 1,925 adult ticks, 1,147 (60%) *Hyalomma*, 463 (24%) *Rhipicephalus*, 138 (7%) *Haemaphysalis*, 106 (5%) *Dermacentor*, and 71 (4%) *Ixodes* ticks were collected from humans (Table 1). The majority of the ticks were from Amasya city, which is the most populated district of the Amasya province.

Seasonal Abundance of Ixodid Ticks Infesting Humans. The seasonal abundance of ixodid tick species in districts of Amasya province was summarized in Table 2. More than 26 and 28% of the total ticks were collected in June and July 2008, respectively, whereas 3% were collected in April, 4% in October, and 0.4% in November. *H. marginatum, H. detritum, H. turanicum*, and *H. aegyptium* were the most prevalent species (Table 1). As

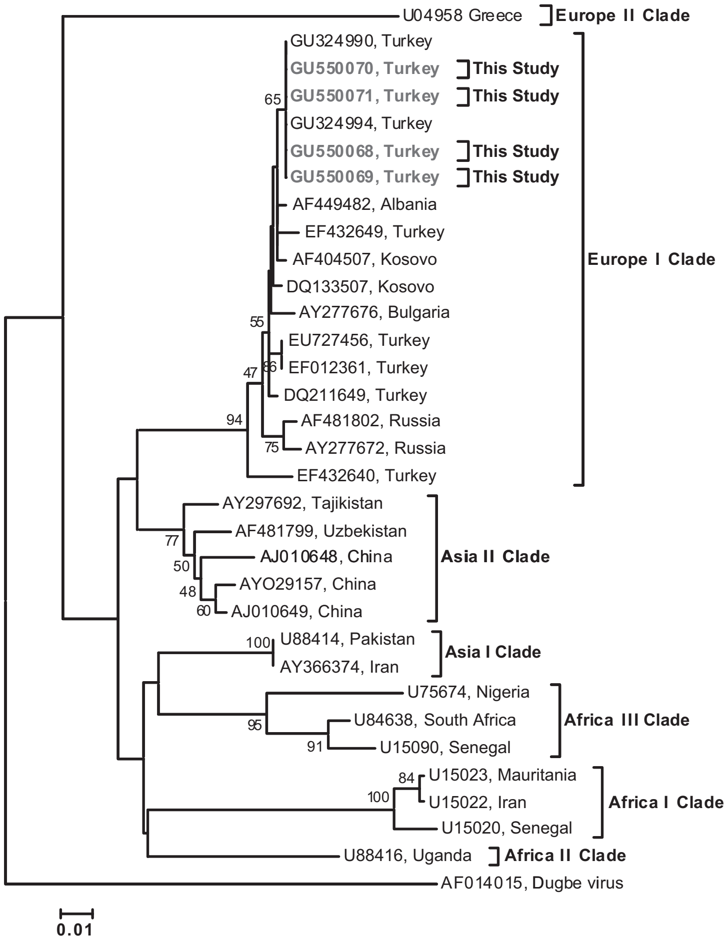

Fig. 3. Phylogenetic relationship of the nucleoprotein gene sequences of CCHFV (obtained from *Hyalomma* ticks collected from Amasya, Turkey) using the neighbor-joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 1,000 replicates (Felsenstein 1985). Branches corresponding to partitions reproduced in <50% bootstrap replicates are collapsed. The bootstrap values (of 1,000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale. The phylogenetic distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. Codon positions included were first + second + third + noncoding. There were a total of 220 nucleotides in the final data set. Phylogenetic analyses were conducted using MEGA4 (Tamura et al. 2007) and BioEdit 7.4.0.1 (Hall 1999). Dugbe virus was used as an outgroup.

shown in Table 2, seasonal abundance of *H. marginatum* gradually increased from 8 to 30% from April to September and declined 2.4% in October. *H. detritum* was higher (17%) in July, whereas abundance of *H. turanicum* was ~10% in June and July. The highest numbers of *H. aegyptium* ticks were observed in June (Table 2).

R. bursa ticks were the most prevalent among other *Rhipicephalus* species, and they were greater in abundance in June and July than some *Hyalomma* species (Table 2). *R. sanguineus* and *R. turanicus* were the most prevalent in June and July. The highest numbers of *Haemaphysalis* and *Dermacentor* species were observed in spring and fall (Table 2). In the *Haemaphysalis* group, *H. parva* and *Haemaphysalis punctata* were the most abundant species, whereas *D. marginatus* and *Dermacentor niveus* were the most prevalent species in *Dermacentor* group (Table 1). Although *Ixodes* species were the least abundant species throughout the year, *I. ricinus* ticks were common especially in April and October (Table 2).

Detection of CCHFV in *Hyalomma* Tick Pools and Phylogenetic Analysis of CCHFV Sequences. In the current study, *Hyalomma* ticks (*H. marginatum*, *H. detritum*, *H. turanicum*, *Hyalomma anatolicum*, and *H. aegyptium*), which are the most common vectors of CCHFV in Turkey, were randomly pooled to make 25 tick pools of 10 ticks without grouping ticks in species. The presence of CCHF virus in tick pools was confirmed by visualizing 536 bp of RT-PCR products amplified using primers specific for CCHFV (Fig. 2). Results showed that 28% of the tick pools (seven pools) were CCHFV positive.

To assess the phylogenetic position of the viral sequences obtained, CCHFV nucleoprotein gene se-

Table 1. Diversity and relative abundance of ixodid tick species infesting humans in Amasya province

Tick species	Districts										*Total*	*Total*
	Amasya		*Merzifon*		*Suluova*		*Gumushacikoy*		*Tasova*		*(% species)*	*(% genus)*
I. redikorzevi Olenev, 1927		5♀		4♀		2♀		1♀		7♀	19 (0.99)	
I. ricinus (Linnaeus, 1758)	2♂	6♀	1♂	6♀		1♀		6♀	3♂	5♀	30 (1.56)	71 (4)
I. laguri Olenev, 1929 sensu Olenev, 1931		3♀		5♀		2♀		6♀		1♀	17 (0.88)	
I. hexagonus Leach, 1815		2♀								3♀	5 (0.26)	
H. aegyptium (Linnaeus, 1758)	16♂	12♀	13♂	9♀	17♂	11♀	7♂	9♀	15♂	8♀	117 (6.08)	
H. anatolicum Koch, 1844	11♂	4♀	16♂	7♀	9♂	5♀	8♂	4♀	12♂	6♀	82 (4.26)	
H. dromedarii Koch, 1844	2♂	1♀	3♂	2♀	2♂	1♀	2♂		3♂	3♀	19 (0.99)	
H. excavatum Koch, 1844	13♂	8♀	8♂	3♀	14♂	4♀	5♂		8♂	9♀	72 (3.74)	
H. detritum Schulze, 1919	19♂	12♀	24♂	13♀	34♂	32♀	27♂	18♀	29♂	25♀	233 (12.1)	1147 (60)
H. rufipes Koch, 1844	1♂		3♂		5♂						9 (0.47)	
H. isaaci Sharif, 1928	13♂		15♂		10♂		18♂		11♂		67 (3.48)	
H. marginatum Koch, 1844	44♂	34♀	42♂	26♀	36♂	21♀	47♂	35♀	41♂	33♀	359 (18.6)	
H. turanicum Pomerantsev, 1946	26♂		23♂		33♂		35♂		39♂		156 (8.1)	
H. franchinii Tonelli & Rondelli, 1932[a]	8♂	6♀	4♂	3♀	6♂				4♂	2♀	33 (1.71)	
R. bursa Canestrini & Fanzago, 1878	36♂	32♀	20♂	12♀	19♂	10♀	24♂	15♀	20♂	10♀	198 (10.3)	
R. sanguineus (Latreille, 1806)	17♂	7♀	14♂	11♀	13♂	9♀	12♂	10♀	8♂	8♀	109 (5.66)	
R. turanicus Pomerantsev, 1936	16♂	10♀	15♂	11♀	14♂	6♀	10♂	10♀	13♂	9♀	114 (5.92)	463 (24)
R. (Boophilus) annulatus (Say, 1821)		24♀	6♂	12♀							42 (2.18)	
H. concinna Koch, 1844		7♀		4♀	3♂			2♀		6♀	22 (1.14)	138 (7)
H. parva Neumann, 1897	12♂	6♀		14♀	3♂	5♀	6♂	11♀	8♂	5♀	70 (3.64)	
H. punctata Canestrini et Fanzago, 1877	5♂	3♀		5♀	3♂	2♀	4♂	2♀	3♂	5♀	32 (1.66)	138 (7)
H. sulcata Canestrini et Fanzago, 1877	6♂								5♂		11 (0.57)	
H. erinacei Pavesi, 1884									3♂		3 (0.46)	
D. marginatus (Sulzer, 1776)	8♂	6♀	8♂	5♀	4♂	3♀	3♂	2♀	6♂	4♀	49 (2.55)	
D. niveus Neumann, 1897	9♂	7♀	7♂	8♀	2♂	1♀	2♂		4♂	3♀	43 (2.23)	106 (5)
D. daghestanicus Olenev, 1929	5♂		3♂				2♂		4♂		14 (0.73)	
Total (♂ ♀)	269♂	195♀	225♂	160♀	224♂	118♀	212♂	131♀	239♂	152♀	1,925	

[a] New species record for Turkish fauna.

quences of viruses originated from different parts of the world, in addition to Turkey's neighbors, were chosen to construct a phylogenetic tree (Fig. 3). As can be seen in Fig. 3, all the viral sequences obtained in the current study fell in the Europe I clade (Papa et al. 2009), which includes Turkish, Kosovo, and Russian strains.

Discussion

In the current study, a survey on ticks was performed from April to November 2008 to determine the species diversity and seasonal activity of ixodid ticks infesting humans in Amasya. The ixodid tick fauna of Turkey is represented by 39 species from six genera (Merdivenci 1969; Ozkan 1978; Ozkan et al. 1988, 1994; Erman et al. 2007; Bursali et al. 2008, 2009). In Turkey, studies on ticks were primarily based on ticks of domestic and some wild animals (Kurtpinar 1954, Ozkan 1978, El-Metenewy and Zayed 1992, Sayin et al. 1997, Yukari and Umur 2002, Tuncer et al. 2004, Mamak et al. 2006).

In contrast to studies on ticks infesting humans in other regions such as South Africa, South America, and Argentina (Horak et al. 2002b, Guglielmone et al. 2006, Nava et al. 2006), there are only two previous reports on tick infestation on humans in Turkey, such as tick biting of humans in Istanbul (Vatansever et al. 2008) and infestation of humans by 24 different ixodid tick species in Tokat province (Bursali et al. 2009, 2010). It is very obvious that tick bite cases of 2,464 in Amasya and 6,000 cases in Tokat were very high in the region. We suggested that increased awareness about the public health importance of ticks because of increased CCHFV cases, greater media attention, and recent environmental changes, including climatic changes that fostered higher densities of ticks, caused such a dramatic increase in the number of reported tick bites in Amasya and Tokat provinces.

In the current study, of 1,925 adult ixodid ticks infested on humans in Amasya province, 26 ixodid tick species were indentified. The diversity of ixodid ticks infesting humans in Amasya was greater than that of many animal species reported in Turkey (Ozkan 1978,

Table 2. Seasonal abundance of ixodid tick species in Amasya province

Tick species	Months [♂ ♀ (%)]								
	April (%)	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
I. redikorzevi Olenev, 1927		1♀ (0.5)	5♀ (1)	6♀ (1.1)	4♀ (1.1)	1♀ (0.7)	2♀ (2.4)		19
I. ricinus (Linnaeus, 1758)	1♂1♀ (3.2)	1♂2♀ (1.4)	1♂4♀ (1)	1♂4♀ (0.9)	1♂10♀ (3)	1♀ (0.7)	3♀ (3.6)		30
I. laguri Olenev, 1929 sensu Olenev, 1931	4♀ (6.5)	3♀ (1.4)	5♀ (1)	2♀ (0.4)	3♀ (0.8)				17
I. hexagonus Leach, 1815		1♀ (0.5)	1♀ (0.2)	2♀ (0.4)	1♀ (1.1)				5
H. aegyptium (Linnaeus, 1758)		15♂11♀ (0.5)	29♂22♀ (10)	7♂9♀ (3)	14♂6♀ (5.4)	3♂1♀ (3)			117
H. anatolicum Koch, 1844	3♂2♀ (8.1)	8♂4♀ (0.5)	20♂9♀ (5.7)	13♂5♀ (3.3)	12♂6♀ (4.9)				82
H. dromedarii Koch, 1844			4♂2♀ (1.3)	5♂2♀ (1.3)	3♂3♀ (1.6)				19
H. excavatum Koch, 1844		3♂1♀ (1.8)	14♂4♀ (3.6)	18♂9♀ (5)	13♂10♀ (6.2)				72
H. detritum Schulze, 1919	4♂ (6.5)	23♂6♀ (13)	25♂24♀ (9.7)	49♂44♀ (17)	26♂22♀ (13)	3♂3♀ (4.4)	3♂1♀ (4.8)		233
H. rufipes Koch, 1844		3♂ (1.4)	5♂ (1)	1♂ (0.2)					9
H. isaaci Sharif, 1928		5♂ (2.3)	20♂ (3.9)	23♂ (4.2)	19♂ (5.1)				67
H. marginatum Koch, 1844	4♂1♀ (8.1)	22♂16♀ (17)	59♂49♀ (21)	60♂36♀ (18)	39♂30♀ (19)	26♂15♀ (30)	2♀ (2.4)		359
H. turanicum Pomerantsev, 1946	6♂ (3.2)	9♂ (4.1)	33♂ (6.5)	55♂ (10)	39♂ (11)	14♂ (10)			156
H. franchinii Tonelli & Rondelli, 1932		4♂ (1.8)	6♂3♀ (1.8)	8♂6♀ (2.6)	4♂2♀ (1.6)				33
R. bursa Canestrini & Fanzago, 1878	1♂ (1.6)	9♂5♀ (6.4)	50♂26♀ (15)	36♂37♀ (13)	20♂11♀ (8.4)	3♂ (2.2)			198
R. sanguineus (Latreille, 1806)		12♂8♀ (9.1)	18♂12♀ (5.9)	21♂16♀ (6.8)	12♂9♀ (5.7)	1♂ (0.7)			109
R. turanicus Pomerantsev, 1936		5♂6♀ (5)	19♂11♀ (5.9)	31♂20♀ (9.4)	139 (5.9)				114
R. (Boophilus) annulatus (Say, 1821)	11♀ (18)	2♂2♀ (1.8)	4♂9♀ (2.6)	9♀ (1.7)	5♀ (1.4)				42
H. concinna Koch, 1844	2♀ (3.2)	4♀ (1.8)	3♀ (0.6)	2♀ (0.4)		5♀ (3.7)	6♀ (7.2)		22
H. parva Neumann, 1897	6♂ (9.7)	3♂8♀ (5)	1♂4♀ (1)	3♀ (0.6)	2♂2♀ (1.1)	9♂11♀ (15)	6♂10♀ (19)	2♂3♀ (71.4)	70
H. punctata Canestrini et Fanzago, 1877	3♂1♀ (6.5)	5♀ (2.3)	2♂ (0.4)		2♀ (0.5)	6♂2♀ (5.9)	4♂7♀ (13)		32
H. sulcata Canestrini et Fanzago, 1877	2♂ (3.2)	3♂ (1.4)				1♂ (0.7)	4♂ (4.8)	1♂ (14.3)	11
H. erinacei Pavesi, 1884						3♂ (2.2)			3
D. marginatus (Sulzer, 1776)	3♂2♀ (8.1)	3♂1♀ (1.8)	1♂ (0.2)		4♂5♀ (2.4)	8♂7♀ (11)	9♂5♀ (17)	1♂ (14.3)	49
D. niveus Neumann, 1897	1♂2♀ (4.8)	2♂3♀ (2.3)	2♂1♀ (0.6)	2♂ (0.4)	3♂2♀ (1.4)	5♂4♀ (6.7)	9♂7♀ (19)		43
D. daghestanicus Olenev, 1929	2♂ (3.2)				4♂ (1.1)	3♂ (2.2)	5♂ (6)		14
Total	62 (3.2)	219 (11.4)	507 (26.3)	542 (28.2)	370 (19.2)	135 (7)	83 (4.3)	7 (0.4)	1,925

El-Metenewy and Zayed 1992, Yukari and Umur 2002, Tuncer et al. 2004, Mamak et al. 2006). Results showed that *H. marginatum, H. detritum, R. bursa, R. sanguineus, H. parva, D. marginatus,* and *I. ricinus* were the most prevalent tick species infesting humans in the region. Of the 26 species identified, *H. franchinii* Tonelli & Rondelli, 1932, was a new record for the tick fauna of Turkey (Table 1). It is really surprising that diversity of tick species (26 of 46 [56.5%]) infested on humans was very high not only in Amasya province, but also other provinces such as Tokat. We proposed that the higher density of ticks may lead to increased infestation of various tick species on humans in the region. According to our unpublished data, tick population is increased especially in Tokat and some other cities around Tokat city in Turkey from 2002 to 2009, and we believe that there is a correlation between increased tick population, tick bite cases, and CCHFV cases between 2002 and 2009. We also suggested that tick fauna of Turkey is not limited to 46 species because there are very limited studies on ticks infesting on rodents, reptiles, birds, and some other wild animals in Turkey. Even though 26 and 24 tick species were found on humans in Amasya (this study) and Tokat (Tekin et al. 2010), respectively, we detected CCHFV in only *Hyalomma* tick pools consisted of a blend of *H. marginatum, H. detritum, H. turanicum, H. anatolicum,* and *H. egyptium* in Amasya as we reported in this study, and in *H. marginatum, H. turanicum, H. detritum, Haemaphysalis concinna, R. bursa,* and *R. turanicus* in Tokat city (our unpublished results). It is possible that some of the other species may contribute to the transmission of CCHFV or other tick-borne pathogens to humans.

Surprisingly, *H. aegyptium* ticks, which usually infest on tortoises, were very common on humans in Amasya, as reported for humans in Istanbul, Turkey (Vatansever et al. 2008), and Tokat province (Bursali et al. 2009). In contrast to higher prevalence of *H. aegyptium* larvae on humans (Vatansever et al. 2008), most of the *H. aegyptium* ticks infesting humans in Amasya and Tokat were in adult stage. It is possible that *H. aegyptium* might be associated with transmission of CCHFV to humans in Turkey, as we detected CCHFV in *H. aegyptium* collected from a hedgehog in Tokat province (Tekin et al. 2009). Results may indicate that accidental infestation of some tick species on humans is possible. However, some tick species might prefer to infest on humans, because we recorded >1,500 (this study) and 6,000 tick bites on humans in Amasya and Tokat (Bursali et al. 2010), respectively.

Results clearly showed that abundance of ticks changed by season, as we found *Hyalomma* and *Rhipicephalus* species were more prevalent especially in summer and declined in the fall (Table 2). High prevalence of these species in general was parallel to a higher incidence of CCHF cases in the summer (Yilmaz et al. 2009, Ergonul 2006), indicating that these species may involve CCHFV transmission in Turkey. In contrast, *Haemaphysalis* and *Dermacentor* species started in April, declined in summer, and increased in fall again. Proportion of *Ixodes* species infesting humans was ≈10% in April and declined by August, indicating that seasonal climatic factors limit abundance of ticks.

Even though various tick species infest on humans in Amasya, information about the presence of tick-borne diseases in ticks and humans is very limited. The cases of tick-borne encephalitis (TBE) and Tularemia were reported in humans by Esen et al. (2008) and Barut and Cetin (2009), respectively. In addition, presence of *Borrellia* species in *H. aegyptium* and *I. ricinus* from western Turkey (Guner et al. 2003), Mediterranean spotted fever (Mert et al. 2006), and Babesiosis (Gun et al. 1996) in humans was reported in Turkey. These results indicate various tick species infesting humans in Amasya might be responsible for the emergence of tick-borne diseases. Emergence of numerous CCHF cases between 2002 and 2008 in Turkey, including Amasya province (Ergonul 2006, Gozalan et al. 2007, CIDRAP 2006, Yilmaz et al. 2009, The Republic of Turkey Ministry of Health 2010a), and detection of CCHFV in several ixodid tick species infesting humans in Tokat (Tekin et al. 2009) may indicate that ixodid ticks play a major role in transmission of CCHFV to humans in the region. Determination of CCHFV in *Hyalomma* tick pools from Amasya in the current study supports previous reports and indicates that various prevalent *Hyalomma* species infesting on humans could act as a potential vector of CCHFV in Amasya and the rest of Turkey.

The exact source and reservoirs of CCHFV in Turkey are not known. Because we detected CCHFV in a *H. aegyptium* tick collected from a hedgehog in Tokat province (Tekin et al. 2009), this may indicate that some small mammals such as hedgehogs and small rodents might be reservoirs of CCHFV in the region. As can be seen in Fig. 3, all the CCHFV sequences obtained in this study fell in the Europe I clade and are identical with GU324490 and GU324494, which are the sequences obtained through our recent report (Tekin et al. 2010) (our unpublished data), confirming the prevalence of this virus in ixodid ticks of Middle Black Sea Region of Anatolia. It is suggested that CCHFV spread from Balkans and Russia to Turkey through migratory birds or uncontrolled transportation of farm animals on the borders, because of a high homology of Turkish CCHFV sequences to some Kosovian, Bulgarian, and Russian CCHFV strains.

In summary, this is the first report on species diversity, seasonal abundance, and CCHFV presence of ixodid ticks infesting humans in Amasya province of Turkey. According to the results presented, a variety of ixodid tick species infests on humans, depending on the season. Detection of CCHFV presence in *Hyalomma* tick pools indicates a possible contribution of the most prevalent *Hyalomma* species, such as *H. marginatum, H. detritum, H. turanicum, H. anatolicum,* and *H. egyptium* to the transmission of tick-borne diseases to humans in Amasya province.

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