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RESEARCH ARTICLE

Predominancy of *Rhipicephalus turanicus* in Tick-infested Sheep from Turkey: a Large-scale Survey

Onur Ceylan[1], Ali Uslu[2], Ceylan Ceylan[1] and Ferda Sevinc[1,*]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Parasitology, Konya-Turkey
[2]Selcuk University, Faculty of Veterinary Medicine, Department of Microbiology, Konya-Turkey
*Corresponding author: fsevinc@selcuk.edu.tr

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ABSTRACT

Ticks are obligatory blood-sucking ectoparasites of a wide variety of animals, and they cause economic losses in livestock breeding by acting as vectors of some bacterial, protozoan and viral pathogens. In the current study, tick-infested sheep was investigated. For this purpose, 4115 sheep from all provinces of Turkey were examined for tick infestation. A total of 1575 adult ticks were collected from 24 different provinces between April and October 2017. Tick infestation rate was determined as 8.8% (n: 362). No argasid ticks were encountered among the collected ticks. Six tick species were morphologically identified belong to Ixodidae, namely as *Rhipicephalus turanicus* (90.86%), *Rhipicephalus bursa* (4.32%), *Rhipicephalus sanguineus* (0.19%), *Hyalomma excavatum* (4.32%), *Hyalomma marginatum* (0.25%) and *Dermacentor marginatus* (0.06%). This study reveals that *Rh. turanicus* is more abundant than the other species causing infestations in sheep, and reports the tick infestation in sheep for the first time in some provinces (Antalya, Ardahan, Aydın, Batman, Bursa, Denizli, Isparta, İzmir, Kahramanmaraş, Kırşehir, Manisa, Muğla, Nevşehir, Uşak, Yalova ve Yozgat) of the country.

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INTRODUCTION

Turkey, consisting of seven geographical regions, is located between longitudes 26-45°E and latitudes 36-42°N, and serves as a wide land bridge between African, Asian and European countries. Due to its geographical location, climate characteristics, animal management systems and numerous marshes and immigrate bird stations, Turkey has favorable conditions regarding the lives and distribution of ticks (Inci *et al*., 2016, Ceylan *et al*., 2021).

As one of the most significant disease-transmitting vectors, ticks transmit several bacterial, viral, rickettsial and parasitic diseases, and it has been reported to transmit nineteen tick-borne diseases (TBDs) (acanthocheilonemasis, aegyptianellosis, anaplasmosis, babesiosis, bartonellosis, *Candidatus* Rickettsia *vini*, Crimean-Kongo Haemorrhagic Fever, cytauxzoonosis, ehrlichiosis, hemoplasmosis, hepatozoonosis, louping-ill, lumpy-skin disease, Lyme disease, tick-borne encephalitis, tick-borne typhus, tick-borne relapsing fever, theileriosis and tularemia) in Turkey (Inci *et al*., 2016).

Sheep breeding is preferred owing to orientation of these animals to the compelling environmental conditions, the ability of reproducing in a short time and their high fertility (Asfaw 1997). As in many other countries (Devendra, 1996; Asfaw, 1997), sheep breeding makes a significant contribution to Turkey's national economy (Ceylan *et al*., 2021). Thanks to sheep farming, most of which are carried out with traditional methods, many people earn their livelihood by selling sheep products and surplus animals (Irshad *et al*., 2010; Ceylan *et al*., 2021). In order to sustain the economic contributions obtained from sheep in a sustainable way, treatment of sheep diseases and taking preventive measures against these diseases play a critical role. Tick infestations and TBDs are the most significant diseases adversely affecting to sheep breeding in Turkey, and cause serious economic losses due to deaths, yield losses and expenditures of diagnosis, control and treatment (Ceylan *et al*., 2021, Ozubek and Aktas, 2017).

The studies conducted in different provinces of Turkey have reported the infestations of *Rh. sanguineus, Rh. bursa, Rh. turanicus, Boophilus annulatus, D. marginatus, D. niveus, H. excavatum, H. anatolicum, H. aegyptium, H. detritum, H. marginatum, Hae. parva, Hae. sulcata, Hae. punctata, Hae. concinna, Hae. inermis, Hae. otophila, I.*

ricinus, Ornithodoros lahorensis, and Argas percicus on sheep (Çakmak *et al*., 1991; Sevinc and Dik, 1996; Emre *et al*., 2001; Yukarı and Umur, 2002; İnci *et al*., 2003; Çiçek *et al*., 2004; Yay *et al*., 2004; Mamak *et al*., 2006; Sayın *et al*., 2009; Yılmaz and Değer, 2011; Arserim and Mete, 2012; Aydın *et al*., 2012; Orkun *et al*., 2016; Ozubek and Aktas, 2017; Eser and Çiçek, 2018; Karataş, 2020).

Detection and treatment of tick infestations are essential to prevent both direct damage of ticks and TBDs caused by attached ticks. For this purpose, more comprehensive understanding and epidemiological surveys on the distribution of ticks involved in pathogen transmission have a crucial role. This study was designed to determine the prevalence of tick infestations adversely affecting Turkey' sheep farming in a wider geographic area.

MATERIALS AND METHODS

Geographic area and sampling: The study was conducted between April and October 2017, and the presence of tick infestation in sheep were investigated in 81 provinces from seven different regions of Turkey. The number of examined animals was calculated by using the stratified sampling method, and the minimum number of animals to be used in the study was determined as 3840 at 95% confidence interval. During the study, 4115 sheep from randomly selected 169 herds from all provinces of Turkey were examined for tick infestation. Detailed information regarding the sampling places, time and the number of visited sheep herds are given (Table 1).

Tick collection and conservation: A total of 1575 adult ticks were collected from 24 different provinces (Aksaray, Anara, Antalya, Ardahan, Aydın, Batman, Burdur, Bursa, Çorum, Denizli, Isparta, İzmir, Kahramanmaraş, Kayseri, Kırşehir, Konya, Manisa, Muğla, Nevşehir, Niğde, Sivas, Uşak, Yalova, Yozgat). Especially the ear, tail and the inguinal regions of sheep were carefully checked for the presence of ticks. Ticks attached to different body parts of sheep were also encountered (Fig. 1). The collected tick specimens were placed in labelled bottles having 70% ethanol under aseptic conditions and transported to Department of Parasitology, Selcuk University, Konya, Turkey for stereomicroscopic identification. While removing the ticks, they were held from the capitulum area with the help of a fine forceps, and capitulum, which contains many morphological details, was removed without detaching it. The number of examined sheep and the provinces with tick infestation are indicated (Fig. 2).

Identification of ticks: The identification of collected ticks was made under stereozoom microscope by examining the external morphological characteristics of ticks by using relevant literature (Walker *et al*., 2014).

Ethical statement: The approval of the owners of sheep was obtained before sampling. All procedures used were applied according to the ethical guidelines for the use of animal samples permitted by Selcuk University, Faculty of Veterinary Medicine (Permit for animal experiment: 2017/36, Date: 27.03.2017).

Fig. 1: Ticks on upper eyelid of sheep.

Fig. 2: The number of examined sheep and the provinces with tick infestation (colored with orange).

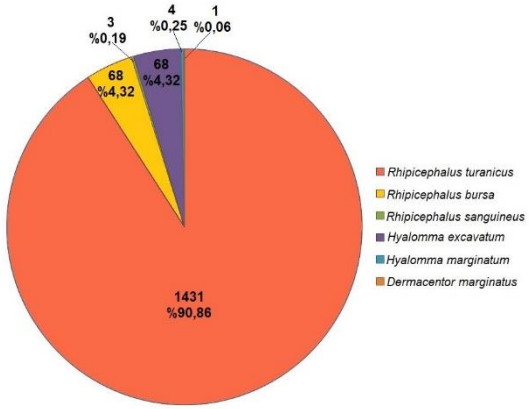

Fig. 3: The distribution of identified ticks.

RESULTS

A total of 1575 adult ixodid ticks were collected from 24 different provinces (Aksaray, Ankara, Antalya, Ardahan, Aydın, Batman, Burdur, Bursa, Çorum, Denizli, Isparta, İzmir, Kahramanmaraş, Kayseri, Kırşehir, Konya, Manisa, Muğla, Nevşehir, Niğde, Sivas, Uşak, Yalova, Yozgat). In the other provinces, no tick infestation was detected in the animals examined during the study. No argasid ticks were identified among the collected ticks. As a result of the stereomicroscopic examination, ticks were identified as *Rh. turanicus*, *Rh. bursa*, *Rh. sanguineus*, *H. excavatum*, *H. marginatum* and *D. marginatus*, respectively. *Rhipicephalus turanicus* was found to be more prevalent than the other ticks identified in the current study. Considering the provinces where ticks were collected, *Rh. turanicus* was found in all provinces except Ardahan. It was also observed that Isparta and Konya provinces have a great tick species diversity. According to the provinces, the distribution of tick species collected from sheep during the research is shown in Table 2.

Rhipicephalus turanicus, *Rh. bursa*, *Rh. sanguineus*, *H. excavatum*, *H. marginatum* and *D. marginatus* were identified at the rates of 90.86, 4.32, 0.19, 4.32, 0.25 and 0.06%, respectively (Fig. 3).

Table 1: Sampling places, time and the number of visited sheep herds in the study

Regions (Provinces)	Tick sample collection time (2017)	Number of visited sheep herd (sheep number)
Aegean Region (Afyon, Aydın, Denizli, İzmir, Kütahya, Manisa, Muğla, Uşak)	April, May	32 (458)
Central Anatolia Region (Aksaray, Ankara, Çankırı, Eskişehir, Karaman, Kırıkkale, Kırşehir, Konya, Nevşehir, Niğde, Sivas, Yozgat)	May, June	26 (763)
Black Sea Region (Western Black Sea subregion; Bartın, Bolu, Düzce, Karabük, Kastamonu, Sinop, Zonguldak)	June, July	12 (155)
Marmara Region (Balıkesir, Bilecik, Bursa, Kocaeli, Sakarya, Yalova)	June, July	12 (267)
Mediterranean Region (Adana, Antalya, Burdur, Hatay, Isparta, Kahrmanmaraş, Mersin, Osmaniye)	July, August	17 (403)
Southeasetern Anatolia Region (Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa, Şırnak)	August	22 (774)
Black Sea Region (Middle/Eastern Black Sea subregion; Amasya, Artvin, Bayburt, Çorum, Giresun, Gümüşhane, Ordu, Rize, Samsun, Tokat, Trabzon)	September	16 (267)
Eastern Anatolia Region (Ağrı, Ardahan, Bingöl, Bitlis, Elazığ, Erzincan, Erzurum, Iğdır, Kars, Malatya, Tunceli, Van)	September	23 (836)
Marmara Region (Thrace subregion: Çanakkale, Edirne, İstanbul, Kırklareli, Tekirdağ)	October	9 (192)
Total		169 (4115)

Table 2: Provinces with tick infestation, infestation rates and the identified tick species

City	n	Number of infestation (%)	*Rh. turanicus*	*Rh. bursa*	*Rh. sanguineus*	*H. excavatum*	*H. marginatum*	*D. marginatus*	♂	♀	Total (%)
Aksaray	50	14 (28.0)	91	-	-	-	-	-	68	23	91 (5.78)
Ankara	71	16 (22.5)	40	1	-	-	-	-	34	7	41 (2.60)
Antalya	64	1 (1.6)	1	-	-	-	-	-	-	1	1 (0.06)
Ardahan	50	1 (1.8)	-	-	-	-	-	1	1	-	1 (0.06)
Aydın	68	19 (27.9)	45	-	-	-	-	-	24	21	45 (2.86)
Batman	95	15 (15.8)	182	-	-	-	-	-	135	47	182 (11.56)
Burdur	40	3 (7.5)	4	-	-	-	-	-	2	2	4 (0.25)
Bursa	45	4 (8.9)	3	1	-	-	-	-	-	4	4 (0.25)
Çorum	30	24 (80.0)	141	-	-	-	-	-	104	37	141 (8.95)
Denizli	95	42 (44.2)	339	-	-	1	-	-	131	209	340 (21.59)
Isparta	62	17 (27.4)	14	14	-	4	2	-	28	6	34 (2.16)
İzmir	65	14 (21.5)	35	-	2	-	-	-	18	19	37 (2.35)
K. Maraş	50	14 (28.0)	34	-	-	-	-	-	11	23	34 (2.16)
Kayseri	70	19 (27.1)	95	-	-	-	-	-	84	11	95 (6.03)
Kırşehir	68	4 (5.9)	5	-	-	-	-	-	1	4	5 (0.32)
Konya	81	40 (49.4)	100	45	-	63	2	-	129	81	210 (13.33)
Manisa	62	32 (51.6)	73	7	-	-	-	-	27	53	80 (5.08)
Muğla	50	26 (52.0)	74	-	1	-	-	-	34	41	75 (4.76)
Nevşehir	49	7 (14.3)	24	-	-	-	-	-	22	2	24 (1.53)
Niğde	60	7 (11.7)	9	-	-	-	-	-	2	7	9 (0.57)
Sivas	60	17 (28.3)	68	-	-	-	-	-	58	10	68 (4.32)
Uşak	20	11 (55.0)	17	-	-	-	-	-	8	9	17 (1.08)
Yalova	21	2 (9.5)	2	-	-	-	-	-	-	2	2 (0.13)
Yozgat	36	13 (36.1)	35	-	-	-	-	-	29	6	35 (2.22)
Other Cities	2753	-	-	-	-	-	-	-	-	-	-
TOPLAM		362	1431	68	3	68	4	1	950	625	1575
(%)	4115	(8.8)	(90.86)	(4.32)	(0.19)	(4.32)	(0.25)	(0.06)	(60.32)	(39.68)	(100.00)

It has been observed that tick diversity is higher in Isparta and Konya compared to the other cities. The presence of *Rh. turanicus, Rh. bursa, H. excavatum* and *H. marginatum* tick species were detected in both provinces.

DISCUSSION

Veterinary and human medicine are threatened by ticks due to their ability to transmit some infectious bacteria, viruses, helminths and protozoa. It is thought that ticks are the most disease-causing blood-sucking arthropods in domestic and wild animals (de la Fuente *et al*., 2008; Batool *et al*., 2019). Hemoprotozoan and rickettsial pathogens transmitted by ticks affect sheep breeding in European countries by causing economically significant diseases (Stuen, 2016). Among these countries, Turkey is an adversely affected country during tick activity seasons (Ozubek and Aktas, 2017). Ovine tick infestations and tick-borne protozoan infections such as babesiosis cause serious damage to the country' livestock economy (Ceylan *et al*., 2021). It is considered that mismanagement and unawareness of sheep owners as to the effects of tick infestations and TBDs have contributed to these losses.

According to the data obtained in the studies conducted in different provinces, the tick species causing infestation in sheep were reported as *Argas persicus, D. marginatus, D. niveus, I. ricinus, Hae. concinna, Hae. otophila, Hae. punctata, Hae. parva, Hae. sulcata, H. aegyptium, H. anatolicum, H. detritum, H. excavatum, H. marginatum, O. lahorensis Rh. annulatus, Rh. bursa, Rh. sanguineus* and *Rh. turanicus* in Adana, Adıyaman, Afyon, Burdur, Çankırı, Diyarbakır, Elazığ, Gaziantep, Kayseri,

Konya, Samsun, Sivas, Şanlıurfa and Van provinces (Çakmak *et al*., 1991; Sevinc and Dik, 1996; İnci *et al*., 1998; Emre *et al*., 2001; Yukarı and Umur, 2002; İnci *et al*., 2003; Yay *et al*., 2004; Çiçek *et al*., 2004; Mamak *et al*., 2006; Aktaş *et al*., 2007; Yılmaz and Değer, 2011; Arserim and Mete, 2012; Orkun *et al*., 2016; Altay *et al*., 2017; Ozubek and Aktas, 2017; Eser and Çiçek, 2018). Apart from these provinces, there are also studies conducted in Aksaray, Bingöl, Mersin and Kütahya provinces to determine the species causing tick infestations in sheep (Sayın *et al*., 2009; İça and Özkan, 2015). Tick infestations caused by *D. marginatus*, *Hae. parva*, *Hae. concinna*, *Hae. punctata*, *Hae. sulcata*, *H. detritum*, *H. excavatum*, *H. marginatum*, *I. ricinus*, *Rh. bursa*, *Rh. turanicus* and *Rh. sanguineus* were encountered in sheep in Bayburt, Bolu, Çorum, Giresun, Kastamonu, Samsun and Tokat provinces in Black Sea Region of Turkey (Aydın *et al*., 2012). In this study, tick infestation was detected in Antalya, Ardahan, Aydın, Batman, Bursa, Denizli, Isparta, İzmir, Kahramanmaraş, Kırşehir, Manisa, Muğla, Nevşehir, Uşak, Yalova and Yozgat provinces for the first time in sheep in Turkey. Tick species identified in the present study are consistent with the tick species detected on sheep in different provinces of Turkey. No different tick species were detected in the study; however, this study is of epidemiological significance due to representing whole country.

The main tick species causing tick infestation in sheep was determined as *Rh. turanicus* (90.86%), followed by *Rh. bursa* (4.32%), *H. excavatum* (4.32%), *H. marginatum* (0.25%), *Rh. sanguineus* (0.19%) and *D. marginatus* (0.06%). It has been observed that the main tick species causing infestation in sheep may vary in Turkey. In the studies carried out by İnci *et al*. (2003), Mamak *et al*. (2006) and Karataş (2020), *Rh. sanguineus*, *D. marginatus* and *Haemaphysalis* spp. were reported to be the dominant tick species of sheep. Besides, many other studies have reported the dominancy of *Rh. bursa* infestation in sheep in Turkey (Orkun *et al*., 2016; Altay *et al*., 2017; Ozubek and Aktas, 2017; Eser and Çiçek, 2018). Predominancy of *Rh. turanicus* determined in this study was found to be consistent with the results of some studies carried out at provincial level (Yukarı and Umur, 2002; Yay *et al*., 2004, Sayın *et al*., 2009). However, the infestation rate of *Rh. turanicus* (90.86%) obtained in this study is much higher than the rates obtained from other studies. An extensive knowledge of geographic distribution of this tick species, one of the main vector of ovine babesiosis and anaplasmosis, would play a significant role to prevent TBDs.

Other tick species detected in the current study have the ability to transmit many infectious agents to animals, especially sheep, and even humans. Among them, *Rh. sanguineus* is a vector of *Coxiella burnetii* and *Babesia ovis*, which cause infections in sheep (Dantas-Torres, 2008; İnci *et al*., 2016). *Rhipicephalus bursa*, known as the main vector of *B. ovis*, plays a role in the transmission of various *Anaplasma* and *Rickettsia* species, especially *Theileria ovis* and *Anaplasma ovis* (Estrada-Pena *et al*., 2017). *Hyalomma excavatum* and *H. marginatum* also play a role in the transmission of *B. ovis*, *T. ovis* and *A. phagocytophilum* (Inci *et al*., 2016; Estrada-Pena *et al*., 2017). *Dermacentor marginatus* was reported to be able to transmit *C. burnetii*, *B. ovis*, *T. ovis* and some *Rickettsia* species to sheep (Bonnet *et al*., 2013). Apart from these pathogens detected in sheep, *D. marginatus* and *H. marginatum* threat humans by transmitting Crimean-Congo Haemorrhagic Fever virus (Inci *et al*., 2016; Estrada-Pena *et al*., 2017). The present study and the studies reporting the tick species which are the vectors of important pathogens causing infections in sheep, actually point out that animals should be protected against tick infestations and tick transmitted infections. In future, conducting more detailed systematic epidemiological investigations will be beneficial in preventing tick infestations and TBDs in sheep.

The number of ticks collected in this study is less than the number determined in some studies conducted in different provinces or regions of Turkey (Yukarı and Umur, 2002; Yılmaz and Değer, 2011; Aydın *et al*., 2012; Arserim and Mete, 2012; Orkun *et al*., 2016; Eser and Çiçek, 2018), but it is higher than the number determined in many studies (Çakmak *et al*., 1991; Sevinc and Dik, 1996; İnci *et al*., 1998; Emre *et al*., 2001; İnci *et al*., 2003; Çiçek *et al*., 2004; Yay *et al*., 2004; Aktaş *et al*., 2007; Sayın *et al*., 2009; İça and Özkan, 2015; Altay *et al*., 2017; Ozubek and Aktas, 2017). Most of the ticks were collected during the summer months when ticks are active. Almost no tick infestations were encountered in the provinces sampled in autumn. It is considered that the number of ticks collected in this study was found to be lower than some studies due to collection of materials from only few areas in summer months when ticks are commonly seen. It is thought that tick control methods applied especially in spring and summer by animal breeders, who are becoming more conscious concerning animal breeding, diseases and taking preventive measures, are also contributing to this situation.

Conclusions: This study is a comprehensive epidemiological study representing whole country to identify the tick species causing infestations in sheep from Turkey. In the study, six different tick species were identified, and *Rh. turanicus* was found as the predominant tick species. The data regarding the geographical distribution of ticks will be beneficial in terms of taking precautions against tick transmitted diseases to animals and humans. Therefore, further systematic and detailed studies on tick infestations and TBDs of different animal species are required. The precise determination of tick species in a region is possible with a long-term and intensive study in a wide geography, in different climatic and seasonal periods.

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