

# Tick bite cases among hazelnut farm workers in Giresun

## Giresun yöresinde fındık işçileri arasında görülen kene tutma vakaları

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**Received/Accepted:** September 07, 2016 / April 04, 2017

**Conflict of interest:** There is not a conflict of interest.

### SUMMARY

**Objective:** Ticks are arthropod vectors of many diseases. The prevalence of Lyme disease transmitted by *Ixodes* is not known in Turkey. The disease is caused by *Borrelia* species and can also be seen in domestic animals. The aim of this study is to identify the ticks, which are collected from the agricultural workers who admitted to hospitals during the hazelnut harvest season between August and September 2015, at species and genus level and investigate whether these ticks vector of *Borrelia* spp.

**Method:** In this study, 152 ticks collected from 134 patients were investigated. Of all samples including larvae and nymphs, 95.24% were collected in September. All patients who admitted with complaints of tick bites were agricultural workers collecting nuts in the gardens at sea level. The ticks were identified at species and genus level with stereomikroskop, hemolymph fluid examined directly by dark-field microscope and cultured in the Borellia Barbour-Stoenner-Kelly (BSK-H) medium.

**Results:** Of all the ticks, 126 (82.9%) were adults, 13 (8.55%) were nymphs and 13 (8.55%) were larvae. Stereoscopic examination of the ticks revealed that 125 (82.2%) of the adults were *Ixodes ricinus* and 1 (0.65%) was *Rhipicephalus sanguineus*. All of the 13 nymphs (8.55%) and 13 larvae (8.55%) were identified as *Ixodes* spp.. All forms of development cycle were detected in the samples and the forms were inconsistent with the expected development cycle. *Borrelia* spp., the agent of Lyme disease, was investigated in the 146 ticks defined as *I. ricinus* and *Ixodes* spp. *Borrelia* spp were detected by dark field microscope in 3 (2.05%) of the 146 *Ixodes* and *Borrelia* spp. were identified in 5 (3.4%) of the cultures.

**Conclusions:** These results demonstrate that Lyme disease can be seen in our region and the most probable vector is *Ixodes ricinus*.

**Keywords :** *Ixodes ricinus*, Lyme disease, *Borrelia* spp.

### ÖZET

**Amaç:** Keneler pek çok hastalığa vektörlük eden arthropodlardır. *Ixodes* cinsi kenelerin bulaştırdığı Lyme hastalığının Türkiye'deki prevalansı bilinmemektedir. Evcil hayvanlarda da görülebilen bu hastalığa *Borrelia* cinsi bakteriler neden olmaktadır. Bu çalışmanın amacı, 2015 Ağustos ve Eylül aylarında fındık hasat mevsiminde kene tutunma şikayeti ile hastanelere başvuran hastalardan izole edilen keneleri cins ve/veya tür düzeyinde tanımlamak ve *Borrelia* spp. vektörü olup olmadıklarını belirlemektir.

**Yöntem:** Çalışmamız, 134 hastadan toplanan 152 kene üzerinde yapılmıştır. Larva ve nimfler dahil örneklerin %95,24'ü Eylül ayında toplanmıştır. Kene tutma şikayetiyle müracaat eden tüm hastalar deniz seviyesindeki bahçelerde fındık toplayan tarım işçilerinden oluşmaktadır. Keneler stereomikroskop ile incelenerek cins ve tür düzeyinde tanımlanmış, hemolenf sıvıları karanlık alan mikroskobu ile direkt olarak incelenmiş ve Borellia Barbour-Stoenner-Kelly (BSK-H) besiyerinde kültürü yapılmıştır.

**Bulgular:** Kenelerin 126 (%82,9)'sı erişkin, 13 (%8,55)'ü nimf ve 13 (%8,55)'ü larva olarak saptanmıştır. Erişkinlerin 125 (%82,2)'i *Ixodes ricinus*, 1 (%0,65)'i *Rhipicephalus sanguineus*, 13 nimf (%8,55) ve 13 larvanın (%8,55) tamamı ise *Ixodes* spp. olarak bulunmuştur. Bütün gelişim formları bir arada ve beklenen döngülerinin dışında saptanmıştır *I. ricinus*

türü ve *Ixodes* spp. olarak tanımlanan kenelerin 146'sında Lyme hastalığı etkeni *Borrelia* cinsi bakteriler araştırılmıştır. *Ixodes* cinsi 146 kenenin 3 (%2,05)'ünde karanlık alan mikroskobu ile *Borrelia* spp görülmüş, 5 (%3,4)'inde ise kültürde *Borrelia* spp. üremiştir.

**Sonuç:** Bu sonuç yöremizde Lyme hastalığının görülebileceğini ve bölgemizde rol alabilecek ana vektörün de *Ixodes ricinus* olabileceğini düşündürmektedir.

**Anahtar sözcükler:** *Ixodes ricinus*, Lyme hastalığı, *Borrelia* spp.,

## INTRODUCTION

Ticks are obligate blood-sucking ectoparasites throughout their development stages and take the second place after the mosquitos among vector arthropods that threaten human health all over the world<sup>1</sup>. Tick species can vary depending on the geographic and climatic conditions such as humidity, precipitation, vegetation and the presence of domestic and wild animals<sup>2</sup>. Subtropical climate zone, which also includes Turkey, is a geography that provides desired conditions for ticks to pursue their biological activities<sup>3</sup>.

It is well known that ticks transmit approximately two hundred different diseases to humans and animals<sup>4</sup>. Overall in Turkey, *Hyalomma* and *Rhipicephalus* spp. are common among ticks collected from human hosts and especially *Hyalomma* spp. often come to the fore because of being the agent of Crimean-Congo Hemorrhagic Fever (CCHF), which may cause severe clinical consequences<sup>2, 4</sup>. Babesiosis, erlichiosis (HGE), tick typhus (rickettsioses), tularemia and filariasis (subcutaneous form) can be considered among the diseases that can be transmitted by *Ixodes* spp.<sup>5</sup>. Tick-borne diseases that affect human and animal health cause significant economic losses worldwide<sup>6, 7</sup>.

The precise prevalence of Lyme disease transmitted through *Ixodes* spp. in Turkey is not known because it is not included in notifiable diseases and can easily be confused with other diseases. The disease is caused by *Borrelia* spp. and can also be seen in domestic animals<sup>8</sup>. *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* and *Borrelia afzelii* are the three genotypes that cause Lyme disease in humans, all being subsumed under the name *B. burgdorferi sensu lato*<sup>9</sup>. Lyme disease caused by the spirochete *Borrelia* genus is the most common zoonotic disease in North America, Asia and Europe<sup>10</sup>.

The data about species and genus of the ticks isolated from patients in Black Sea Coast Region and infective agents that are transmitted through these ticks are missing. In this study, the ticks collected from the agricultural workers who admitted to emergency departments in the nut-collecting season in summer were identified at

species and genus level and it was investigated whether these ticks vector *Borrelia* spp.

## MATERIAL AND METHODS

A total of 152 ticks (126 adult + 13 nymph + 13 larvae) collected from 134 nut farm workers who admitted to emergency department between August and September 2015 were included in the study.

The ticks collected from the patients were placed in screw capped plastic containers 100/15 mm in size. Small holes were previously opened in these tubes for venting and green leaf pieces were placed in order to provide appropriate humidity. All the ticks collected from the same patient were placed in a separate tube and the information about the date and the place that the ticks were collected were noted on these tubes. In this way, the ticks were transported to the laboratory and they were identified at species level by using stereomicroscope (Olympus SZX10) when they were alive<sup>11-13</sup>. Six of the ticks were excluded from the study since they were not alive during the laboratory examination. A total of 146 *Ixodes* genus ticks were placed in first petri filled with sterile distilled water and kept there for 3-5 minutes and then were taken to the second petri containing 70% ethyl alcohol. After 3-5 minutes in the second petri, the ticks were placed in the third petri again filled with sterile distilled water and kept there for 3-5 minutes. The ticks were then placed on a sterile slide and the tail of each tick was dissected by a sterile scalpel in order to take the hemolymph and digestive system out by pushing the body of the tick gently. The collected part was crushed in physiological serum and covered with lamellae in order to search for *Borrelia* spp. via dark-field microscope (Zeiss AX10) at 200X and 400X magnification. Barbour-Stoenner-Kelly (BSK-H) medium was used for the culture of the organism. The information about the regions from where our patients collected nuts were also noted down in our study.

## RESULTS

Of all ticks, 126 (82.9%) were adults, 13 (8.55%) were nymphs and 13 (8.55%) were larvae. Of all the collected ticks, 99.35% were identified as *Ixodes ricinus* and *Ixodes* spp. Of 126 adults, 125 (82.2%) were identified as *Ixodes ricinus* (♀) and

1 (0.65%) was identified as *Rhipicephalus sanguineus* (♂). All of the 13 nymphs (8.55%) and 13 larvae (8.55%) were found as *Ixodes* spp (Table 1). All the adult ticks that are identified as *Ixodes ricinus* were female (Figure 1). Throughout the study period, 95.24% of the samples including larvae and nymphs were collected in September. All forms of development cycle were detected in

the samples and the forms were inconsistent with the expected cycles. These ticks were examined directly by dark-field microscope and cultured in the Borellia Barbour-Stoenner-Kelly medium. *Borrelia* spp. were detected by dark field microscope in 3 (2.05%) of the 146 *Ixodes* and *Borrelia* spp were determined in 5 (3,4%) of the cultures.



**Figure 1.** The female *Ixodes ricinus*

**Table 1.** The tick species collected from hazelnut farm workers in emergency department

Species	Male n (%)	Female n (%)	Larvae n(%)	Nymphs n (%)	TOTAL
<i>Ixodes ricinus</i>	-	125 (% 82.2)	-	-	125
<i>Ixodes spp.</i>	-	-	13 (% 8.5)	13 (% 8.5)	26
<i>Rhipicephalus sanguineus</i>	1 (% 0.6)	-	-	-	1
TOTAL	-	126 (% 82.8)	13 (% 8.5)	13 (% 8.5)	152

All the patients admitted to hospital with tick bite complaint were agricultural workers collecting nuts in our city center or sea-level county (Table 2).

**Table 2.** Distribution of farm workers with tick bites by hazelnut agricultural land

Agricultural Lands	(n)	%
Giresun City Center	23	17.1
Bulancak	22	16.4
Kesap	27	20.1
Espiye	11	8.2
Eynesil	14	10.4
Gorele	14	10.4
Piraziz	13	9.7
Tirebolu	10	7.4
TOTAL	134	100

## DISCUSSION

Lyme disease is the most common vector borne infection in the United States. It mostly effects the northeast region and the number of cases is increasing every year. The incidence of the disease in Europe is higher in the northern and central regions<sup>14</sup>. The first case in Turkey was isolated from the Black Sea region<sup>15</sup>. In some areas, the disease was also detected in wild and domestic animals. Despite the presence of seroprevalence studies in various animals and narrow-scoped human case reports, comprehensive epidemiological studies are not available in Turkey<sup>9, 16, 17</sup>.

*Ixodes* spp. are the most important vectors that play role in the spread of Lyme disease. The most known vectors are *Ixodes pacificus* in the Western Pacific part of North America, *Ixodes scapularis* in the northeast and midwest of North America, *Ixodes ricinus*, also known as the sheep tick, in Europe and *Ixodes persulcatus* in Asia [18]. In our study, 125 (82.23%) of the identified adults were *Ixodes ricinus*. All of the 13 nymphs (8.55%) and 13 larvae (8.55%) were identified as *Ixodes* spp. (Table 1).

Researchers<sup>19, 20, 21</sup> demonstrated that *Ixodes* spp. are generally found more intensely in moist and temperate regions. Aydin et al.<sup>20</sup> reported that *Ixodes ricinus* is found in a small number in the domestic animals of western Black Sea Region. They also stated that it has never been observed in

Corum, Tokat and Bayburt that are the cities located upcountry and do not have temperate climate of the Black Sea. In the same study<sup>20</sup>, the highest *Ixodes ricinus* detection rate was in Giresun among seven cities included in the study. All of the ticks (99.35%) except one sample were identified as *Ixodes* spp. in our study and this finding is consistent with the outcomes of the above mentioned study. Moreover all the patients in our study were nut farmers working in the city center or sea-level county which have temperate climate (Table 2).

In the two previous studies that Güner et al. presented<sup>22, 23</sup>, it was reported that spirochetes of the genus *Borrelia* were detected in the 95.8% and 38.7% of the *Ixodes ricinus* ticks collected via flag method from the timberland in Trakya and Sinop, respectively. In addition, Lyme seropositivity rate was detected to be 6 to 35.9% among agricultural workers<sup>24</sup>. In our work, *Borrelia* spp. were detected by dark field microscope in 3 (2.05%) of the 146 *Ixodes* spp. and *Borrelia* spp were determined in 5 (3.42%) of the cultures. These results demonstrate that Lyme disease can be seen in our region and the most probable vector is *Ixodes ricinus*.

All adults that were identified as *Ixodes ricinus* in our study were female. This can be explained by the fact that the adults complete mating before holding on to the host.

*Ixodes ricinus* is a three-host tick and can infest all wild and domestic mammals, reptiles and

humans<sup>12</sup>. The unique features of Black Sea climate such as high rainfall, moisture and vegetation structure allow the presence of this tick intensely in this region.<sup>20,21</sup>. It was reported that the activity of the ticks increase as from November, reach the most intense activity in April and May and decrease excessively between July and October in the Black Sea coast of İstanbul<sup>20</sup>. In the Mediterranean region, Estrada Pena et al.<sup>12</sup> demonstrated that the adult ticks are active from October until the middle of March while the larva and nymph forms generally show activity between April and July. Selcuk et al.<sup>25</sup> could not determine any case in Bursa in June-August and reported the least common period that these ticks are seen to be September. They detected the active larvae and nymphs between March and July. On the other hand, inconsistent with these studies, Kar et al.<sup>26</sup> reported that all forms of *Ixodes ricinus* are active throughout the year in the Black Sea coast of Istanbul.

Although our study was performed in August-September, three different development forms of *Ixodes ricinus* were collected from the patients. The vast majority (95.24%) of the samples, including larvae and nymphs were collected in September. Dominance of the adult ticks (82.23%) suggests the high level of activity in these month. Current findings were considered to be in parallel with the results of Kar et al.<sup>26</sup>. This fact is considered to arise from the generation of *Ixodes ricinus* twice or more in a year due to our province's climate features. Aydın and Bakırcı<sup>21</sup> also reported that the possible increase in temperature and rainfall can increase the tick population dramatically resulting in spread of the Lyme diseases

## CONCLUSIONS

Consequently, our work is a preliminary study that has been made for the first time in our region. Since the region is narrow and nut harvest season is quite short, the number of samples is limited. However, identification of 99.35% of the ticks as *Ixodes ricinus* and *Ixodes* spp. and determination of all development forms together and inconsistently with the expected development cycle are important findings. The high ratio (17%) of the larvae and nymphs which are expected to be rarely present in the hosts during the study period and the high overall Lyme carriage rate (3.42%) in the ticks are also worth reporting. Additionally misdiagnosis of Lyme is conceivably when the local community and the physicians are not informed properly about the tick-borne diseases. In our region, more comprehensive and organized surveillance studies

are planned by screening domestic animal hosts along with the tick bite cases in order to determine vectorial features of the ticks.

## Acknowledgement

This study was presented as poster presentation in 3. National Microbiology Congress (18-22 November, 2015)

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