EJMO

DOI: 10.14744/ejmo.2017.36854
EJMO 2017;1(3):124-128

Research Article

Epidemiological Distribution of Ticks Analyzed at Our Laboratory between April 2008 and November 2015; A Study of *Borrelia Burgdorferi* in Ixodes

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Abstract

Objectives: The aim of this study was to determine the distribution of ticks in the Marmara region and the ratio of *Borrelia burgdorferi* infection caused by ticks (*Ixodes spp*).

Methods: Live ticks collected from the Marmara region were brought to the laboratory. Ticks on patients who came to our laboratory with tick bites were removed using forceps. These ticks were examined by stereo microscope and identified. The patients were informed about ticks and tick-borne diseases. People bitten by *Hyalomma* ticks were directed to the infectious diseases clinic and were kept under observation. The presence of *B. burgdorferi* in these ticks was determined using dark-field microscopy and *B. burgdorferi* was cultured in vitro using Barbour-Stoenner-Kelly (BSK)-H medium.

Results: Of the 462 ticks in our sample, 208 (45%) belonged to the genus *Ixodes*, 105 (22.7%) to *Boophilus*, 63 (13.6%) to *Rhipicephalus*, 61 (13.2%) to *Hyalomma*, 20 (4.3%) to *Dermacentor*, and 5 (1.1%) to *Haemaphysalis*. Ixodes was the most prevalent genus of ticks observed, and 194 of the 208 (93.3%) were *I. ricinus*. Patients diagnosed with Lyme disease were treated. *Borrelia* was observed in 4 (2.1%) *I. ricinus* ticks from Istanbul and Kastamonu using dark-field microscopy, and 6 ticks (3.1%) contained *Borrelia* that reproduced in the BSK-H medium. There was no clinical development of Crimean-Congo hemorrhagic fever among the individuals under observation.

Conclusion: Tick-borne Lyme disease does not receive the public attention that CCHF has, but our results indicate that it is an important public health problem.

Keywords: *Borrelia*, *ixodes*, lyme disease, tick

Cite This Article: *Polat E, Sirekbasan S, Gokalp H, Kutlubay Z. Epidemiological Distribution of Ticks Analyzed at Our Laboratory between April 2008 and November 2015; A Study of Borrelia Burgdorferi in Ixodes. EJMO. 2017; 1(3): 124-128*

Ticks are the most important group in the Acarina family. They are the most dangerous parasites in the world, following mosquitoes, due to the pathogenic impacts and damage they cause to humans and animals. Turkey's subtropical climate and geographical location create an ideal environment for the development and reproduction of ticks. Widespread animal husbandry and uncontrolled movement of pets and wild animals in Turkey add to the difficulty of controlling tick populations. Different types of ticks can spread in certain zoogeographic zones and are carried between countries by migratory birds, traded goods, commodities, and various forms of transportation. Control of ticks is problematic, as efforts made are neither ordered nor controlled. Male and female ticks subsist on the blood of vertebrate animals, living as temporary ectoparasites during the larvae, nymph, and adult periods of

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Submitted Date: June 20, 2017 **Accepted Date:** September 22, 2017 **Available Online Date:** September 29, 2017

their life cycle. Ticks destroy tissue and can cause allergic reactions and paralysis due to the toxic effects of substances in their saliva. Furthermore, ticks are capable of infecting humans and other animals with diseases such, as Lyme, Rocky Mountain spotted fever, Mediterranean fever, Tularemia, Brucellosis, and Babesiosis.[1-5] Ticks became popular in written and visual media during the 2002 outbreak of Crimean–Congo hemorrhagic fever (CCHF) in Turkey. Thus, there has been a considerable increase in the number of incoming patients who fear they are at risk of developing tick-borne illnesses. The media has exaggerated the issue, and fear of ticks has been heightened in the society. This has occurred to such a degree that patients have requested the surgical removal of skin tags that have been mistaken for ticks. Despite these exaggerated fears, ticks are vectors of many diseases besides CCHF.

We have diagnosed individuals in the Black Sea and Marmara regions with Lyme disease caused by *Ixodes ricinus* bites. *I. damini*, *I. pasificus*, and *I. scapularis* have been found to cause Lyme disease in America, *I. ricinus* in Europe, *I. persilcatus* in Asia, and *I. ovatus* in Japan.[6] Lyme disease was identified for the first time in Turkey in 1990 by Cakir et al. and Polat et al. published two reports on Borrelia infections caused by *I. ricinus* bites in Ordu-Unye and Bartin in 1998.[7,8] They published three cases of Lyme disease, which were positively identified by culturing the blood of infected individuals in 2010, for the first time in Turkey.[9]

In this study, we aimed to investigate the epidemiological distribution of ticks and the rate of *Borrelia* carrying *I. ricinus* in different regions of Turkey.

Materials and Methods

This study was conducted between April 2008 and November 2015, and ticks were collected from the Marmara region by field scanning. Collected ticks were placed in plastic tubes that were 100/15 mm in size with a screw lid. Small air holes were made in these tubes and green leaves were placed inside the tubes to provide an environment for the ticks to hold on to that also had the proper humidity. A maximum of 10 ticks were placed in a tube and the location and date of tick collection was recorded on the tube.[7] Ticks were brought to the laboratory alive and examined. Ticks on people who arrived at our laboratory were removed with forceps. Ticks that were removed at different health institutions from bitten individuals via different methods were also brought to our laboratory. Ticks removed from people in the Mediterranean, Black Sea, and eastern and south-eastern anatolia regions were also sent to our laboratory. These ticks were examined under a stereo-microscope (Olympus SZX10) and their types were identified. [2] Ticks of *Ixodes spp*. were placed in a petri dish with sterilized distilled water and were incubated for 3–5 min. Then, they were taken in a second petri dish with 70% ethylene alcohol and incubated for 3–5 min. They were then transferred into a third petri dish

Table 1. The number and types of ticks according to provinces

Types of Ticks	Province							Total	%
	Istanbul	Mugla	Kırklareli	Kocaeli	Bartın	Tekirdag	Hakkari		
I. ricinus	132	9	3	-	11	-	3	158	38.8
I. hexagonus	5	-	-	-	-	-	-	5	1.2
I. laguri	6	-	-	-	-	-	-	6	1.5
I. frontalis	2	-	-	-	-	-	-	2	0.5
B. kohlsi	29	34	-	-	-	14	-	77	19.0
B. annulatus	18	8	-	-	-	-	-	26	6.4
R. bursa	26	12	-	-	-	7	-	45	11.1
R. sanguineus	5	4	-	-	-	-	-	9	2.2
R. turanicus	3	2	-	-	-	-	-	5	1.2
H. marginatum	17	1	-	2	12	-	-	32	7.9
H. aegyptium	5	-	-	-	-	-	-	5	1.2
H. plumbeum	4	-	-	3	-	-	-	7	1.7
H. dromedarii	2	-	-	-	-	-	-	2	0.5
H. otophila	-	-	2	-	-	-	1	3	0.7
D. marginatus	6	6	-	-	-	-	-	12	2.9
D. niveus	4	4	-	-	-	-	-	8	2.0
H. punctata	3	-	-	-	-	-	-	3	0.7
H. numidiana	2	-	-	-	-	-	-	2	0.5
Total	269	80	5	5	23	21	4	407	100

which had sterilized distilled water and were incubated for 3–5 min. After this process, the rear end portion of the ticks were removed using a sterile lancet, and their digestive tract with hemolymph were resected.[8-12, 13] This portion of the tick was then crushed in saline, covered with lamella and *Borrelia* was visualized with dark-field microscopy (Zeiss AX10) at 200× and 400× magnification. *Borrelia* spp. were also cultured using Barbour–Stoenner–Kelly (BSK-H) medium.[7]

Results

The types and numbers of ticks sent to our laboratory from Istanbul, Muğla, Kırklareli, Kocaeli, Bartın, Tekirdağ, and Hakkari are indicated in Table 1.

A total of 462 ticks were examined; these included *Boophilus kohlsi* from Balıkesir (2 ticks), *I. ricinus* from Bolu (17 ticks), *Rhipicephalus bursa* from Bursa (2 ticks), *I. ricinus* from Sakarya (2 ticks), *I. ricinus* from Yalova (5 ticks), *I. ricinus* from Kastamonu (2 ticks), *I. ricinus* from Ordu (5 ticks), *I. hexagonus* from Samsun (1 tick), *Hyalomma aegyptium* from Çorum (2 ticks), *H. aegyptium* from Bayburt (2 ticks), *H. marginatum* from Tunceli (3 ticks), *H. marginatum* from Bingöl (2 ticks), *I. ricinus* from Germany (2 ticks), *I. ricinus* from the Netherlands (3 ticks), and *R. sanguineus* from the USA (2 ticks). A total of 675 R. bursa were counted in transparent plastic bags containing chemicals that were imported from France.

Of the 462 ticks in our sample, 208 (45%) belonged to the genus *Ixodes*, 105 (22.7%) to *Boophilus*, 63 (13.6%) to *Rhipicephalus*, 61 (13.2%) to *Hyalomma*, 20 (4.3%) to Dermacentor, and 5 (1.1%) to *Haemaphysalis*. Ixodes was the most prevalent genus of ticks, comprising 208 ticks, of which 194 (93.3%) were *I. ricinus*.

Following the removal of ticks that bit a woman and a girl in the Istanbul Belgrad forest and a male in Kastamonu, redness and swelling developed 15–20 days later at the site of the bite (Figs. 1, 2). Patients diagnosed with Lyme disease were treated. Borrelia was observed in four (2.1%) of the I. ricinus ticks from Istanbul and Kastamonu using dark-field microscopy (Fig. 3) and six ticks (3.1%) contained *Borrelia* that reproduced in the BSK-H medium (Fig. 4).

Conclusions

Ticks became popular in the written and visual media in Turkey after the 2002 outbreak of CCHF disease. Therefore, a severe irrational fear of ticks has been fostered among Turks. Highlighting this point, people have increasingly sought surgical intervention for skin tags, thinking they are ticks (Fig. 5).

CCHF was first described in "Congo" in 1945. The Nazis conquered Crimea in 1940, and they prohibited the public from hunting. In 1945, the Red Army expelled the Nazis

Figure 1. Lyme disease.

Figure 2. A tick of *Ixodes ricinus* type.

Figure 3. Borrelia spp. isolated from *Ixodes ricinus* (Original 400x).

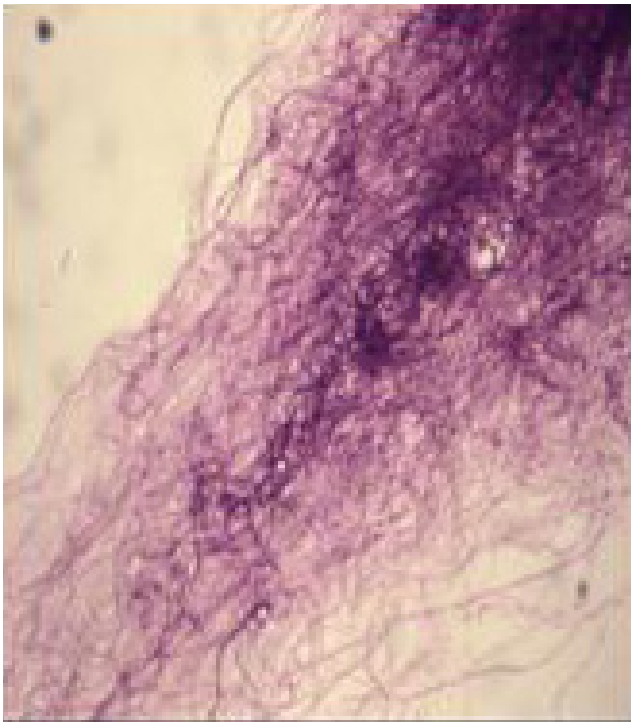

Figure 4. Borrelia culture in a BSK-H medium.

from Crimea and assisted Crimeans in harvesting agricultural products. This resulted in the infection of 200 soldiers with tick-borne illnesses, of whom 20 died.[14] The view on this issue of the Ecologist is that CCFH emerged as a result of the increased number of ticks due to return to natural wildlife. CCFH was then seen in Turkey in 2002 after many years.[7] However, while Lyme disease was first detected in 1975 and *B. burgdorferi* was identified as the pathogen responsible for causing Lyme disease in 1982, Lyme disease was first reported in Turkey in 1990.[8-10, 11]

CCHF is endemic to 30 countries worldwide and can be

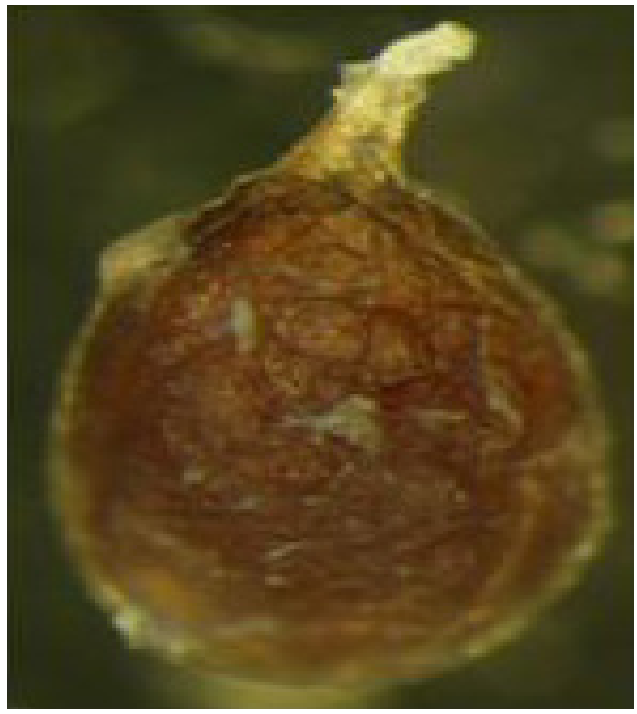

Figure 5. Skin tag removed from a patient.

found in all countries that border Turkey. Mass culling of poultry in response to bird flu epidemic is a source of CCFH in Turkey, as has been reported in Congo. This is due to a considerable increase in the number of ticks reported after mass culling of poultry. Due to the lack of winged animals that prey on ticks, ticks that entered shelters on non-winged animals were able to access houses and hotels where they infected people. While it is known that unprotected contact with infected live or dead poultry can transmit bird flu to humans, there are no reported cases of bird flu transmission between humans. Despite this, it is possible that the virus could gain the ability to infect via human-to-human transmission.

Based on the events above, people have started to bring the ticks into our laboratory with great fear after 2002, and there has been an increase in the number of studies that investigate tick-borne diseases. While the majority of these studies have focused on CCHF virus-carrying *Hyalomma* ticks, it is known that ticks can transmit several other infectious diseases.[15-17]

Ixodes were the most numerous with 208 (45%) ticks, of which 194 (93.3%) were *I. ricinus*. Redness and swelling appeared approximately 15–20 days at the site of tick bites after two women were bitten in the Istanbul Belgrad forest and a man was bitten in Kastamonu (Fig. 1). All three patients were diagnosed with Lyme disease and were ap-

propriately treated. *Borrelia* was observed in four (2.1 %) I. ricinus from Istanbul and Kastamonu (Fig. 2) and six ticks (3.1%) contained Borrelia reproduced in the BSK-H medium (Fig. 3).

The genera *Ixodes* 208 (45%), *Boophilus* 105 (22.7%), *Rhipicephalus* 63 (13.6%), *Hyalomma* 61 (13.2%), *Dermacentor* 20 (4.3%), and *Haemaphysalis* 5 (1.1%) were determined.

Removal of the whole tick including hypostome from people who were bitten by ticks is critical. The entry of the hypostome creates a route of infection through the skin when it remains in the skin, and presence of the hypostome can result in local edema, allergies, and sometimes severe allergic reactions.

Several methods of tick removal are used in different health care institutions, such as surgery, tying a string to the tick, vacuuming the tick, and using alcohol. However, holding the tick using forceps as close to the skin as possible and pulling it perpendicular to the skin is the easiest way to remove a tick[6]. Surgical removal of ticks is painful for the patient and is time-consuming for the physician. Therefore, healthcare providers should be well informed about the removal of ticks.

Ticks that were removed from people were examined using a stereo-microscope and were defined. People were informed about ticks and tick-borne diseases. People who were identified as having been bitten by *Hyalomma* ticks were directed to the infection clinic and were kept under observation. There was no clinical development of CCHF among individuals under observation. Individuals determined to have been bitten by *Ixodes* ticks were instructed to go to the laboratory if symptoms such as redness, burning, and itching occurred. Following these instructions, three people who were bitten by *I. ricinus* in Kastamonu and Istanbul Belgrad Forests were later diagnosed with Lyme disease and were accordingly treated. Although these results are not in the agenda like CCHF, they do indicate that tick-borne Lyme disease is an important public health concern.

Disclosures

Ethics Committee Approval: This study was conducted in accordance with local ethical rules.
Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship contributions: Concept – E.P., S.S., Z.K.; Design – E.P., G.K., Z.K.; Supervision – E.P., Z.K.; Materials – E.P., S.S.; Data collection &/or processing – E.P., G.K., Z.K.; Analysis and/or interpretation – E.P., Z.K.; Literature search – E.P., S.S., Z.K.; Writing – E.P., Z.K.; Critical review – E.P., Z.K.

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