

Full Length Research Paper

## Isolation and identification of tick borne bacterial pathogens in Turkey and Iraq

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The aim of this study was to detect the pathogenic bacteria from hard ticks collected from animals and soil in Turkey and Iraq. For this, 195 adult hard ticks were identified according to the taxonomic keys. A total of 195 hard ticks were identified by as 149 *Hyalomma* spp. and 46 *Rhipicephalus* spp. The diagnosis of tick borne pathogens was made by microbiological techniques. One hundred sixty four (164) bacteria were isolated from the ticks. These bacteria were as follows: 119 *Escherichia coli*, 18 *Salmonella* spp., 10 *Klebsiella* spp., 9 *Serratia* spp., 6 *Shigella* spp., and 2 *Enerobacter aerogenes*. The study investigated tick borne pathogens affecting humans and animals. These pathogens are transmitted by different hard ticks' species. The ticks and tick borne diseases are an important public health problem in the world.

**Key words:** Hard ticks, *Hyalomma* species, *Rhipicephalus* species, tick borne pathogens.

### INTRODUCTION

Hard ticks are obligate blood-feeding ectoparasites which transmit a greater variety of pathogenic microorganisms and are the cause of significant economic losses (Ostfeld et al., 2006). There is great variation in the dominant tick species in different regions of the United States (Merten and Durden, 2000; Stromdahl and Hickling, 2012). Each species of human-biting hard ticks is a vector for a different suite of infectious agents. They can carry different pathogens, and cause important human

diseases (Parola and Raoult, 2001). There are several important pieces of hard tick's life cycle. First, at each of the feeding times, obtaining a blood meal is essential for the tick's survival. If the tick fails to find a host at any of these important developmental times, it does not enter the next stage of development and dies as a result; and this has interesting implications for vector control. If one of the host populations were theoretically wiped out, the ticks would not be able to obtain a blood meal and would

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therefore die. Another thing to note about the life cycle is that each tick feeds on different animals, but humans can be infected at any of the feeding stages of a tick. So although ticks generally feed on domestic and wild animals, they are able to feed on humans and that facilitates disease spread (Heaney, 2012).

Hard ticks contain 702 species in 14 genera (Barker and Murrell, 2004). In this study, Genus *Hyalomma* and Genus *Rhipicephalus* were identified. The Genera are common in Asia, Europe, and Africa. These hard ticks are of medical, veterinary, and economic importance because they are the vector of a lot of these pathogens. They transmit the pathogens that cause the animal and human diseases such as, east coast fever, anaplasmosis, babesiosis, rickettsiosis, boutonuse fever, Lyme disease, Q-fever, Rocky Mountain spotted fever, and Crimean-Congo hemorrhagic fever, and several bacterial pathogens (Olwoch et al., 2007).

The purpose of this study was to isolate and identify the pathogenic bacteria from hard ticks collected from Turkey and Iraq.

## MATERIALS AND METHODS

### Design and samples collection

This study applies a descriptive method. One-hundred and ninety five (195) samples of adult ticks were collected from animals and soil from Iraq (Duhok city and Sinjar town) and Turkey (Kahramanmaraş city) between the period from April to June 2014. The adult ticks were collected from the back leg and tail regions of the animals and were sent to Kahramanmaraş Sütçü İmam University Medicine Faculty Research Laboratory for the determination of species and for pathogen analysis.

### Microbiological and parasitological analysis

For this, 195 ticks were identified under the stereo microscope according to the taxonomic keys and classification criteria (such as capitulum, palp, festoon, cervical canal, anal shield, accessorial shield, and scutum colour) (Dumler and Rosen-Feld, 2000; Walker et al., 2003). Each tick was washed in sterile salty water (0.85% NaCl) and later their outside sections were disinfected with 70% ethyl alcohol, and they were washed in salty water again. Each tick was placed into tubes with sterile distilled water. Then the tubes were homogenized with the homogenizer (Daihan HS-30E, DAIHAN Scientific, Korea) at 3000 rpm for 3 min and then were centrifuged at 4000 rpm for 30 min for extraction of supernatants (Arıkan et al., 2009; Stojek and Dutkiewicz, 2004). Each supernatant was inoculated onto 5% sheep blood agar (Merck, Germany) and endo agar (Merck) agar. Then each culture was incubated in aerobic and anaerobic media at 37°C for 24-48 h. The cultures were evaluated with routine microbiological methods (Dumler and Rosen-Feld, 2000; Koneman et al., 2006).

## RESULTS AND DISCUSSION

A total of 195 hard ticks (149 *Hyalomma* spp. and 46 *Rhipicephalus* spp.) were collected from 90 Iraq (46.15%)

and 105 Turkey (53.84%) and were collected from different sources like sheep, dogs, tortoises and soil (Table 1, Figure 1). We identified the two genera of the hard ticks by using microscope, according to the external features, presence or absence of festoon, cervical canal, anal shield, accessorial shield, and scutum colour (Figure 2). Our results show that, out of 195 samples which were collected 164 (84.10%) were positive for bacterial growth while 31 (15.89%) samples negative. We isolated six species of bacteria which were characterized and belong to the family of Enterobacteriaceae. The species were *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Shigella* spp., *Enerobacter aerogenes* and *Serratia* spp. Distribution of pathogenic microorganisms isolated from the hard ticks are shown in the Table 2.

The *Hyalomma* spp. bites cause stress and blood loss to the hosts. A few ticks are usually well tolerated by livestock and pets, but infestations with dozens or hundreds of ticks can significantly weaken affected animals and cause weight loss, reduced fertility, and decreased milk production. Several characteristics of the ticks make them abeyance vectors of pathogenic agents. Their wide host range and tendency to feed on several hosts during their life cycle ensures ample opportunity to acquire and transmit pathogens. They have a high reproductive potential, ensuring maintenance of large populations and a high frequency of host-vector contact (Brown, 2005).

The *Rhipicephalus* spp. is the most widespread tick in the world and recognized as a vector of many pathogens affecting dogs, sheep and humans. This tick can be found animals living in both urban and rural areas, being highly adapted to live within human dwellings and being active throughout the year not only in tropical and subtropical regions, but also in some temperate areas (Dantas-Torres, 2010).

These hard ticks represent the most important group of arthropod vectors for wild and domestic animals and also to human; they transmit a wide spectrum of pathogenic microorganisms such as viruses, bacteria, and protozoa (Uilenberg, 1995; Jongejan and Uilenberg, 2004).

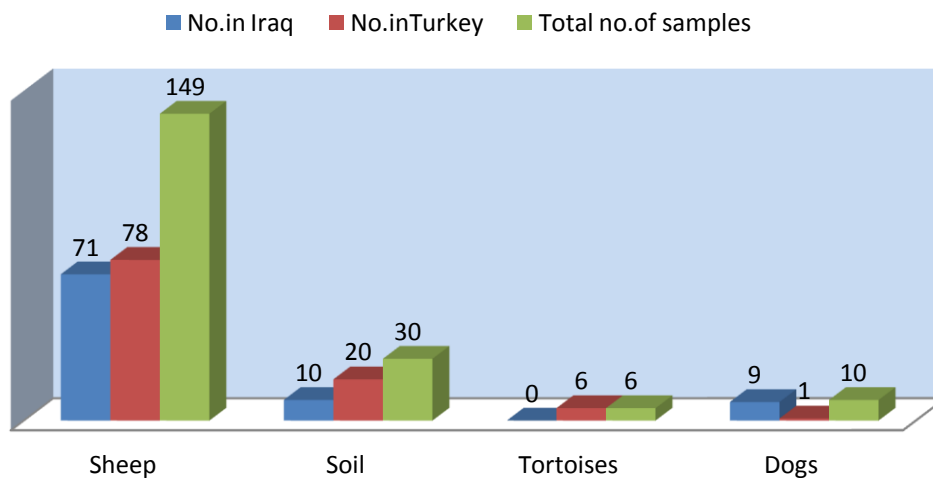
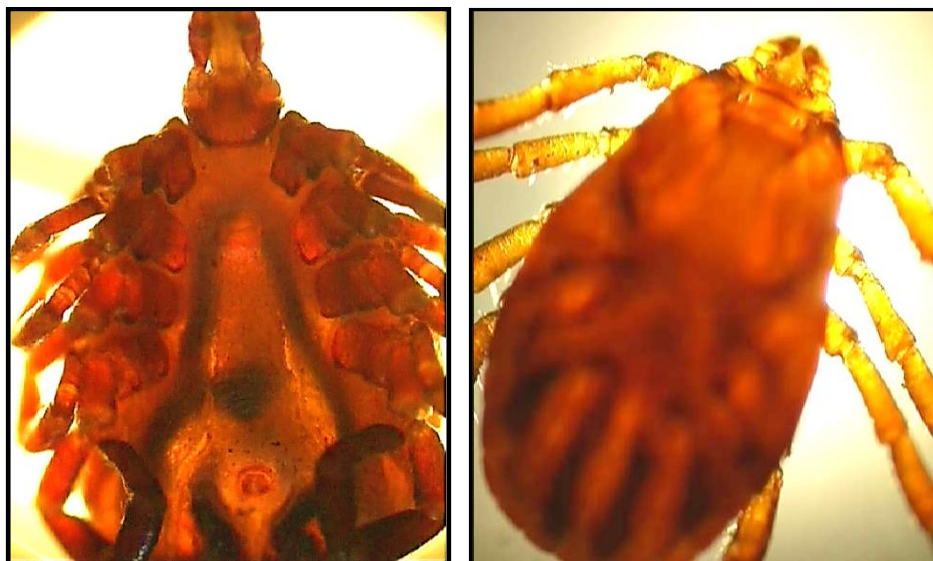
In our study, the most commonly isolated bacterium from several species of hard ticks was *E. coli* (72.56%). In consistence with our results, *E. coli* was the most commonly isolated bacteria from sheep in a study in Basra, Iraq (Mohanad and Moaed, 2012). Their study included isolation of ticks from ear, tail and udder of 60 sheep.

The tick samples were identified and assigned to type *Hyalomma* spp. depending on diagnostic characters which included; being festoons or none, legs appeared like banded and shape of spiracle like long coma in male, triangular shape in female with like tail inside at the end.

In 2013, we had conducted another study which showed *Bacillus* spp. as the most commonly isolated pathogen from *Hyalomma* spp. collected from tortoises (Kirecci et al., 2013). In a study, total of 169 ticks, 35

**Table 1.** Distribution of males and females of the genera of hard ticks.

Genus	Females: n (%)	Males: n (%)
<i>Rhipicephalus</i> spp.	25 (54.34%)	21 (45.65%)
<i>Hyalomma</i> spp.	21 (14.09%)	128 (85.90%)

**Number and sources of collected samples in Iraq and Turkey****Figure 1.** Distribution of the samples of hard tick.**Figure 2.** *Hyalomma* spp. (left, adult female, ventral view) and *Rhipicephalus* spp. (right, adult male, dorsal view).

*Haemaphysalis parva*, 30 *Hyalomma marginatum*, 28  
*Rhipicephalus turanicus*, 25 *Dermacentor marginatus*,

17 *H. excavatum*, 16 *H. aegyptium*, 6 *Hyalomma* spp.  
(nymphs), 3 *Ha. punctata*, 3 *Rh. bursa*, 3 *Rh.*

**Table 2.** Distribution of pathogenic bacteria isolated from hard ticks.

Isolated pathogens from hard ticks	Genera of hard ticks	
	<i>Rhipicephalus</i> spp. n (%)	<i>Hyalomma</i> spp. n (%)
<i>Escherichia coli</i>	22 (13.41%)	97 (59.15%)
<i>Salmonella</i> spp.	8 (4.88%)	10 (6.09%)
<i>Klebsiella</i> spp.	3 (1.83%)	7 (4.27%)
<i>Serratia</i> spp	3 (1.83%)	6 (3.66%)
<i>Shigella</i> spp.	1 (0.61%)	5 (3.05%)
<i>Enterobacter aerogenes</i>	1 (0.61%)	1 (0.61%)
Total = 164 (100%)	38 (23.17%)	126 (76.83%)

*sanguineus*, 2 *Ixodes ricinus*, and 1 *Haemaphysalis* spp. (nymph), were collected from humans in different parts of Ankara, in Turkey (Orkun et al., 2014). In our study, a total of 195 hard ticks, 149 *Hyalomma* spp. (adult) and 46 *Rhipicephalus* spp. (adult) were collected from sheep, dogs, tortoises and soil in Kahramanmaraş city of Turkey and in Duhok city of Iraq. A surveillance study that lasted 4 years in the US categorized 66. 000 types of ticks and *Rickettsia* spp., an important pathogenic bacterial species, were identified in these ticks (Merten and Durden, 2000).

Hard ticks can be vectors of human diseases such as CCHFV, tick paralysis, and Lyme disease. In our study, viruses and unculturable pathogens were not investigated, however, the bacteria we determined are very important pathogens for humans; *Salmonella* spp. and *Shigella* spp. can cause gastroenteritis in humans, while other species may lead to sepsis and various soft tissue diseases.

The prevalence of zoonoses and other infections have been increasing because of expanding human migrations and global climate change. Tick borne pathogens are particularly dangerous in countries with a temperate climate and serious preventive measures should be taken against these infections.

This study agrees with the study of Heaney (2012) since his study shown the importance of ticks in transferring diseases to humans and animals and this importance include the three important steps; first, they can feed on a host animal that is infected and ingest the pathogen in the host's blood. Second, ticks can perform transovarial transmission for most diseases. This means that mothers can pass on the pathogen to their offspring. Third, co-feeding, or multiple ticks feeding at once on the same host, can cause infection. Thus, ticks can carry diseases at every point of their lives and each time a tick needs a blood meal, humans are at risk for catching the disease.

Ticks of the genus *Hyalomma* spp. are known as a vector of viruses such as Congo-Hemorrhagic Fever (CCHFV). Although many species are not involved in disease transmission, the considerable length of

*Hyalomma* spp. mouthparts provokes a painful bite. One of the most important diseases transmitted by this ticks is CCHFV which occurs throughout vast area of Africa, Asia and Europe, but can cause high mortality (Sadek et al., 2007).

To conclude, hard ticks are the most dangerous arthropod which impend or threaten health of vertebrates and they are capable of transmitting the greatest variety of pathogens for both humans and animals and the riskier of hard ticks lies through their feeding manner that makes them important in the health of domestic animals and humans.

### Conflict of interest

Authors have not declared any conflict of interest.

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