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Isolation and characterization of pathogenic bacteria from *Rhipicephalus spp*. adult female hard tick

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Abstract

Ticks are blood-sucking arthropods established in basically all global regions of the world. Around the world, there are about 900 species of ticks. *Ixodid* or hard ticks are made about 700 species, while 200 species are soft ticks. Ticks act as vectors of a wide range of disease agents, including viruses, bacteria, and protozoa. It is well documented that more than 100 000 diseased conditions in a human being in the world are associated with the tick-borne infection. This study intended to isolate some enterobacteriacae (*Escherichia coli* and *Salmonella spp*) from live adult female *Rhipicephalus spp* hard tick. Sixty-seven ticks were collected from cattle and sheep during the period extended from November 2015 till March 2016 from Baghdad governorate. Totally, there were 15 (22.38%) samples revealed bacterial isolates out of 67. The number and percentage of *E. coli*, *Salmonella spp* and both *E. coli* and *Salmonella spp* isolates were 10 (14.92 %), 2 (2.98 %) and 3 (4.47 %) respectively. Moreover, the results showed significant ($P \leq 0.05$) difference between the isolated bacteria. In conclusion, this study approved the isolation of *Escherichia coli* and Salmonella spp from adult female *Rhipicephalus spp* hard tick. The author recommends taking a prevention precaution to control the distribution of ticks that have serious roles in the transmission and distribution of bacterial diseases in domestics animals.

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Introduction

Ticks consider as among the most important vectors of animal (domestic and wild) and human diseases such as protozoa, rickettsiae, bacteria, viruses and helminths of vertebrates. They rank second only to the mosquitoes as vectors of life-threatening or debilitating human and animal diseases. Besides, ticks transmit a greater variety of

infectious agents than any other arthropod group (Andreotti *et al.,* 2011). The cattle tick (*Rhipicephalus*) hinders livestock production in tropical and subtropical parts of the world, where it is endemic (Grisi *et al.,* 2002).

There are various effects of the ticks on the livestock. The first one is the direct economic loss that associated with blood feeding during infestation. While, the second is the indirect effect, where ticks act to transfer the diseases like Bovine Babesiosis and Anaplasmosis. *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* respectively, are the causative agents of these diseases and ticks are the primary source of infection (De la Fuente *et al.,* 2008).

At the beginning of the 20th century, Cowdry, (1925) reported the association between non-pathogenic bacteria and ticks. However, this relation was limited with the pathogenic species. A new microbial association between bacterial communities and ticks has been reported including those that was recognized as unknown tick-borne pathogens or vector competencies previously (Burgdorfer *et al.,* 1973; Clay *et al.,* 2008 and Vilcins *et al.,* 2009).

The understanding the relationship between tick and tick-borne pathogens increase as a result of the progress in the research on tick biology and ecology (Parola and Raoult, 2001). It was well known that the knowledge regarding the ticks borne infectious diseases was increased. Moreover, some tick-borne bacterial diseases were considered an emerging infectious threat globally (Hotez and Kamath, 2009 and Heyman *et al* ., 2010).

Bacteria are ubiquitous microorganisms, and some of them have developed symbioses with ticks. Ticks have the ability to transmit pathogenic bacteria included the genera *Borrelia, Rickettsia, Francisella, Ehrlichia, Anaplasma*, and *Coxiella*. They might have the relationships (commensal, mutualistic, or parasitic) with their tick hosts (Noda et al., 1997 ;Sacchi *et al.,* 2004 ;Scoles, 2004).

The review of literature showed limited research that approved the role of ticks in the transmission of bacterial organisms. Consequently, this study was designed to approve the role of *Rhipeciphalus spp* hard ticks in the transfer of bacterial pathogens such as *E. coli* and *Salmonella spp*.

Materials and Methods

Samples collection

Sixty-seven engorged live adult female hard ticks (*Rhipicephalus spp*) infested cattle and sheep, were collected at a period from November 2015 tillMarch 2016. The tick is embedded its mouthparts into the skin of the animal. The procedures of removing of tick should be done carefully. A reliable method was used to collect the ticks by grasping them as close as possible to animal's skin, with a pair of tweezers and pull firmly away from the skin while rocking it back and forth gently. Then the ticks were kept in clean containers containing 70 % ethyl alcohol (Murrell *et al* ., 2003) and send to Laboratory/ Unit of zoonosis diseases/ college of veterinary medicine / Baghdad University for further investigations. The ticks were classified and dissected under a

septic condition for bacterial isolation. *Escherichia coli* and *Salmonella spp*) isolations.

Bacterial Isolation

A swab was collected from each dissected tick and inoculated onto blood agar. Then, this agar was incubated for 24 hours at 37˚C. All isolated colonies were inoculated on another three agar bases (MacConkey, eosin methylene blue, and Salmonella-Shigella agar). All cultures were incubated at 37 ˚C for 24 hours. The biochemical tests (citrate, methyl red, Indol, and TSI) were done for all isolates according to Quinn *et al.,* (2004).

Statistical Analysis

The data were collected and analyzed by using (SPSS) , and *Chi*-square test was used for comparison at significant level of $P \leq 0.05$.

Results

Two types of *Enterobacteriaceae* were isolated from 15 (22.38 %) out of 67 live adult female hard ticks (*Rhipicephalus spp*) samples. The number and percentage of *E. coli, Salmonella spp* , and both *E. coli* and *Salmonella spp* isolates, were 10 (14.92 %), 2 (2.98 %) and 3 (4.47 %) respectively. Moreover, the results showed significant ($P \leq 0.05$) difference between the isolated bacteria (Table.1).

Discussion

Ticks consider as obligate ectoparasites of vertebrates. The most importance of ticks is its ability to transmit a broad spectrum of pathogenic microorganisms, such as *rickettsiae*, *protozoa*, *spirochaetes*, and viruses. The most common tick-borne pathogens are the protozoan disease such as theileriosis and babesiosis and rickettsial diseases. However, there were only scarce studies that approved the ability of ticks to transmit the bacterial pathogens. Previous studies focused on the isolation of pathogenic bacteria from ticks infested vertebrate hosts such as cattle and sheep (Murrel *et al.,* 2003; Rahman and Rahman, 1980; Brum and Teixeira,1992). Murrell *et al.,* (2003) reported that *Stenotrophomonas, Staphylococcus, Pseudomonas, Acinetobacter* and *Bacillus* were the most common genera of bacteria isolated from ticks, lice, and fleas. The previous study reported that ticks have a hematophagous habit, host body fluids, and attaching at predilection sites (Amoo *et al;*1987). The biological nature of the ticks and its blood feeding nature lead to increase the diversity of microorganisms in the ticks (Heise *et al.,* 2010). Rahman and Rahman, (1980) isolated *Enterobacter, Pseudomonas*, and *Staphylococcus,* from homogenates of *Rhipicephalus microplus* in Bangladesh. Other researchers have also reported the same observations for the closely related tick species *R. decoloratus* and *R. geigyi* in

Africa. However, Amoo *et al* ., (1987) isolated *Staph. aureus* from the hemolymph of adult females and their eggs.

The isolated bacteria were gram negative, rods with rounded ends and non-spore forming. A pure growth of the bacteria was positive for indol and methyl red, while it revealed negative reaction for citrate and voges-proskaur (VP). The colonies of *E. coli* isolates appeared as pink/ lactose fermenting and metallic green sheen on MacConkey's and eosin methylene blue (EMB) agar respectively. Meanwhile, the *Salmonella spp* colonies appeared pale or colorless with the center of black precipitate due to H2S production in Salmonella-Shigella agar. (Figure .1 & 2). The results of the present study disagree in the infection rate with other reported study (Kirecci *et al.,* 2015). Kirecci *et al.,* (2015), were identified 149 *Hyalomma spp.* and 46 Rhipicephalus spp out of 195 hard ticks. Microbiological techniques were made for the diagnosis of tick-borne pathogens. 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*.

Table.1: Shows the bacterial isolates (*E. coli and Salmonella spp*) from the *Rhipicephalus spp* female ticks.

Bacterial Isolates	Number of Samples Examined	Positive	Percentage (%)
E. coli	67	*10	14.92
Salmonella sp.		2	2.98
E. coli and Salmonella sp.		3	4.47
Total		15	22.38

***P ≤ 0.05**

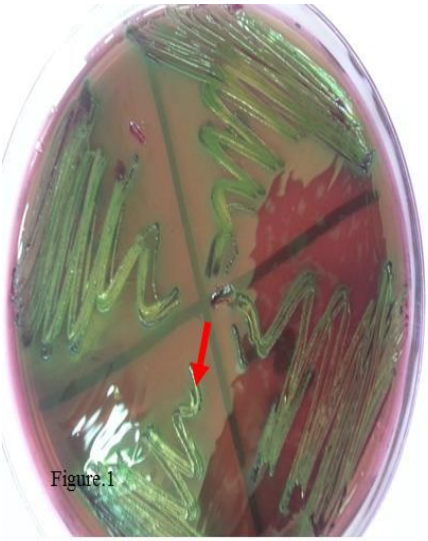

Figure. 1: Shows colonies of *E. coli* on Eosin methylene blue agar (Metallic sheen).
Figure. 2: Shows colonies of *salmonella spp* on SS agar (H2S production)

The variation in the results of the current study with the previous studies might be related to ecological factors. Ticks have special behavior that facilitates contact with bacteria in the environment. It expands the complexity of bacterial communities residing on a tick's exoskeleton. The tick has a particular mechanism which enables the tick to act as a bridging vector. This mechanism facilitates the transmission of microbes across vertebrate hosts and possibly influencing ecological and evolutionary aspects of their natural history (Andreotti et al., 2011).
Moreover, the differences in colony forming unit could be due to the variations in the environmental contamination and season. Moreover, environmental pollution, season, and fodder used for feeding the animals lead to make a difference in percentage distribution of animals having bacteria (Shahzad *et al* ., 2013).
Transovarially transmission acts to enable the bacterial colonization in the initial life cycle" of the ticks. The ticks population and egg fertilization could augment bacteria-tick associations through possibly infected sperm or the microbiota (Afzelius *et al.*,1989). Also, the environment, where the eggs are deposited, influences the type of bacterial communities (Hendry and Rechav, 1981).

In conclusion, the results of this study approved the ability of hard ticks *Rhipicephalus spp* to act as a vector for the *Enterobacteriaceae.* Both Escherichia coli and *Salmonella spp* were isolated from ticks with variation in the isolation rates that depend on ecological factors. The author recommends taking a prevention precaution to control the distribution of ticks that have active roles in the transmission and distribution of bacterial pathogens to their host.

References

Afzelius BA, Alberti G, Dallai R, Godula J and Witalinski W. (1989). Virus- and *Rickettsia* infected sperm cells in arthropods. Journal of Invertebrate Pathology. 53:365-377.

Amoo A O, Dipeolu OO, Akinboade AO and Adeyemi A. (1987). Bacterial isolation from and transmission by *Boophilus decoloratus* and *Boophilus geigyi*. Folia. Parasitol. 34:69-74.

Andreotti R, Pérez de León AA, Dowd SE, Guerrero FD, Kylie G, Bendele KG and Scoles GA.(2011). Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus) microplus* through tag-encoded pyro sequencing. BMC Microbiol.11:6

Brum JGW and Teixeira MO. (1992). Acaricidal activity of *Cedecea lapagei* on engorged females of *Boophilus microplus* exposed to the environment. Arq.Bras. Med. Vet. Zoot. 44:543-544.

Burgdorfer W, Brinton LP and Hughes LE (1973). Isolation and characterization of symbionts from the Rocky Mountain wood tick, *Dermacentor andersoni*. J. Invert. Pathol. 22:424-434.

Clay K, Klyachko O, Grindle N, Civitello D, Oleske D and Fuqua C. (2008). Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. Mol. Ecol. 17:4371-4381.

Cowdry EV. (1925). A group of microorganisms transmitted hereditarily in ticks and apparently unassociated with disease. J. Exp. Med. 41:817-830.

De la Fuente J, Estrada-Peña A, Venzal JM, Kocan KM and Sonenshine DE. (2008). Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosc. 13:6938-6946.

Grisi L, Massard CL, Moya-Borja GE and Pereira JB. (2002). Impacto econômico das principais ectoparasitose sembovinos no Brasil. A HoraVeterinária. 21:8-10.

Heise SR, Elshahed MS and Little SE.(2010). Bacterial diversity in *Amblyomma americanum* (*Acari: Ixodidae)* with a focus on members of the genus *Rickettsia*. J .Med. Entomol. 47:258-268.

Hendry DA and Rechav Y.(1981). Acaricidal bacterial infecting laboratory colonies of the tick *Boophilus decoloratus* (Acarina: Ixodidae). J. Invertebr. Pathol. 38:149-151.

Heyman P, Cochez C, Hofhuis A, van der Giessen J, Sprong H, Porter SR, Losson B, Saegerman C, Donoso-Mantke O. Niedrig M and Papa A. (2010). A clear and present danger: tick-borne diseases in Europe. Expert. Rev. Anti. Infect. Ther. 8:33-50.

Hotez PJ and Kamath A.(2009). Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. PloS .Negl. Trop. Dis; 3:e412.

Murrell A, Dobson SJ, Yang X, Lacey E and Barker SC.(2003). A survey of bacterial diversity in ticks, lice and fleas from Australia.Parasitol. Res. 89: 326–334.

Noda H, Munderloh UG and Kurtti TJ.(1997). Endosymbionts of ticks and their relationship to Wolbachia spp. and tick-borne pathogens of humans and animals. Appl. Environ. Microbiol. 63:3926-3932.

Parola P and Raoult D.(2001). Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clin. Inf. Dis. 32:897-928.

Quinn PJ, Carter ME, Markey B and Carter GR.(2004). Clinical Veterinary Microbiology. 6th ed. Mosby An imp Wolf, London: 13 –17.

Rahman MH and Rahman MM.(1980). Occurrence of some bacterial isolates in ticks found in Madhupur forest area. Bang. Vet. Jour. 14:43-47.

Sacchi L, Bigliardi E, Corona S, Beninati T, Lo N, Franceschi A. (2004). A symbiont of the tick *Ixodesricinus* invades and consumes mitochondria in a mode similar to that of the parasitic bacterium *Bdellovibrio bacteriovorus*. Tissue Cell. 36:43-53.

Scoles G A. (2004). Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. J. Med. Entomol. 41:277-286.

Shahzad KA, Muhammad K, Sheikh AA, Yaqub T, Rabbani M, Hussain T, Anjum AA and Anees M. (2013). Isolation and molecular characterization of shiga toxin producing *E. coli* O 157. J. Anim. Plant Sci. 23(6): 1618-1621.

Vilcins IE, Fournier P, Old JM and Deane E.(2009). Evidence for the presence of *Francisella* and spotted fever group *Rickettsia* DNA in the tick *Amblyomma fimbriatum* (Acari: Ixodidae), Northern territory, Australia. J. Med .Entomol. 46:926-933.