

Identification of the bacterial pathogens in tick (*Rhipicephalus spp.*) infested Iraqi cows

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Abstract: The current article is a preliminary study in Iraq, aimed to shed light on the extent of identification of tick species and bacterial pathogens that found in the ticks. For this purpose, one-hundred eighty local breed cows belonged to a heard in Abojrabe (White Golden Village) were examined during a period extended from May till september 2017. The ages of the cow were ranged from (2-4) years and weighing were (300-350) kg. Ticks were carefully collected from whole body regions. The results of this research revealed that a total number of ticks infestation were 65 (36.12%)out of 180 cows. The ticks identification were *Rhipicephalus spp.* Furthermore two types of bacteria were isolated from (28) (43.08%) live adult female ticks (*Rhipicephalus spp.*) samples. The numbers and percentage of *E. coli* were 16 (57.14%), *Salmonella spp.* 5 (17.86 %) and both *E. coli* and *Salmonella spp* were 7 (25%).

In conclusion the tick infestation in cows pose a serious damage to the livestock industry and it is a challenging task for the workers to control them. The isolation rate of bacteria from ticks might reflect the active contribution of this arthropod in environmental contamination and increase the probability of transmitting bacterial pathogens to their hosts.

Keywords: Iraq, *Rhipicephalus spp.*, Tic, Bacterial pathogens, Cows

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I. Introduction

Ticks, regarded as the largest non-microscopic ectoparasites, are the most important vectors that infest animals, transmit a wide range of pathogens from infected to non-infected hosts. All the dairy animal species are apt to tick infestation. Because of voracious habit of parasite's blood sucking; loss of blood for their rapid development impoverishes the hosts [1]

Rhipicephalus microplus is the most important ectoparasites of cattle and a highly invasive tick species, spreading to all tropical and subtropical regions of the world [2] and causing enormous economic losses to the livestock industry [3] In cattle, tick infestation alone can cause anemia, stress, reduction in weight gain and milk yields, hypersensitivity and toxicities [4,5]. Initially, this parasite is primarily a dangerous parasite of cattle, but it can also be found in horses, sheep, goats and dogs. Some tick species can act as vectors of pathogens causing a number of tick-borne diseases, a serious impairment to cattle health and productivity [6]. Consequently, this study was designed to highlight the presence of bacterial pathogens such as *E. coli* and *Salmonella spp.* in live ticks infested Iraqi cow.

II. Materials and methods

II.1. Tick collection and identification

One-hundred eighty local breed cows belonged to a heard in Abojrabe (White Golden Village) which located west to Baghdad-Iraq. The ages of the cows were ranged between (2-4) years which were estimated on the basis of the dentition score [7] and weighing were (300-350) kg. The entire body surface of the animal was examined thoroughly for the presence of any live tick and all visible adult ticks were collected during a period extended from May to September 2017. Ticks (n= 1300) out of (65) cows were gently picked up by hands using wetted cotton after careful examination of the entire body surface of the cows including ear, neck, dewlap, abdomen, udder, anus, tail and vulva. Ticks from each cow were kept in clean containers containing 70% ethanol [8] then labeled with information on the host (i.e., sample number, age), and date of sampling then send to Laboratory/ Unit of Zoonosis Diseases/ College of Veterinary Medicine/ Baghdad University for further investigations. Ticks were classified in the Natural History Museum in Baghdad according to the method mentioned by [9] and dissected under a septic condition (bacteriological hood) [10] for bacterial isolation a sterile phosphate buffer solution was added to dissected tissues of ticks to avoid dryness.

II.2 Bacterial culture

A swabs were collected from the removed tick extract and inoculated onto blood agar. at 37 °C for 24 in order to maintain and reanimate the weak microorganisms and repair the injured bacterial cells. All isolated colonies were inoculated on another three agar bases (MacConkey, eosin methylene blue and *Salmonella-Shigella* agar) and they were incubated at 37 °C for 24h. The isolated bacteria were classified by using multiple biochemical tests (citrate, methyl red, Indol, and TSI) according to the technique used by [11].

II.3 Statistical analysis

The data collected during the course of study was subjected to statistical analysis. For this purpose, computer software program “SPSS version” 16 were used and calculated the results of data by applying chi square test. Significance was set at the level $P \leq 0.05$.

III. Results

In the present study, a total of 180 cows were examined. The results of this investigation revealed that a total positive of ticks infested cows were 65 (36.12%). The visible female adult ticks that were collected from different body parts of local cows were (n= 1300). In addition out of (65) infested cows, there were 28 (43.08%) infected ticks with two types of bacteria.

The numbers and percentage of *E. coli* were 16 (57.14%), *Salmonella spp.* 5 (17.86%) and both *E. coli* and *Salmonella spp.* were 7 (25%). Moreover, the results showed significant differences ($P \leq 0.05$) between *E. coli*, *Salmonella spp.* and both types of isolated bacteria but lack of significance were recorded between *Salmonella spp.* and both types (Table.1)

The isolated bacteria in our study were (*E. coli* and *Salmonella spp.*) (figures 2 and 3) which are gram negative, rods with rounded ends and non-sporeforming. 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. Shows *Rhipicephalus*.

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

Bacterial isolates	Number of infected ticks	Positive	Percentage
<i>E.coli</i>	28	*16	57.14 %
<i>Salmonella</i>		5	17.86%
<i>E. coli and salmonella</i>		7	25%
Total		28	38.62

* Refers to significant differences ($P \leq 0.05$) between the isolated bacteria.

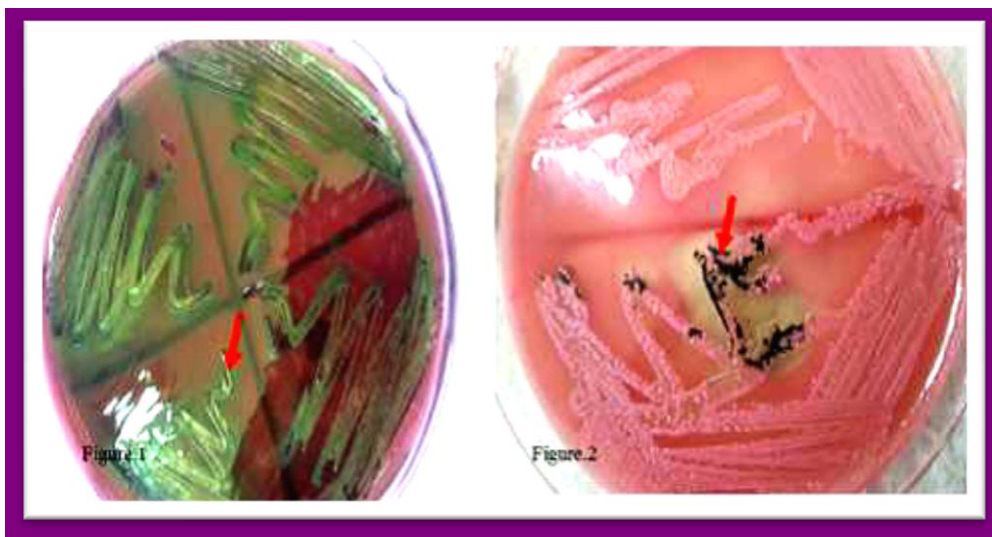


Figure- 2.(left) Shows colonies of *E. coli* on Eosin methylene blue agar (Metallic sheen).

Figure-3. (right) Shows colonies of *salmonella spp.* on SS agar (H₂S production).

IV. Discussion

Ticks are the known to have high medical and veterinary importance. The distribution and abundance of ticks species infesting cattle vary greatly from one area to another. Our study deals with the distribution and prevalence of ticks in cows in the study area. In the present study *Rhipicephalus spp* was found to be the most prevalent tick species with infection rate of (36.12%) this has also been reported as one of the common tick of cattle by other workers [12].

This is the first effort to conduct study in the area. Only single species of tick *Rhipicephalus spp* was identified from the examined livestock of the area. The identified species of tick was also reported by [13] from other areas. Results of the present study are somewhat similar or different to the studies carried out earlier in other part of the world [14]. This further indicated that the finding of this tick in the area of current study is in line with its wide spread occurrence in most parts of the country [15], Several environment related factors which support tick survival in the specific area including; temperature, humidity, rainfall [16], host availability, season, breed, age, sex, stage of lactation, gestation period and nutritional status of the animal [17], body condition method of application of a acaricides [18] and husbandry practices [19], and animal movement [20]. The reason of higher prevalence of ticks in livestock species of the study area could be due to the climate (as the study was performed during summer season) in which the temperature reach to highest degree at this season in Iraq as the climate variables significantly affect ticks distribution in particular region.

Ticks are potential vector of viral, bacterial, protozoa and helminthes diseases with zoonotic significance in animals and human [21], So in this regard evidence about tick prevalence in any specific area in essential for the planning of control measure towards ticks and tick borne diseases.

The results of this study reflected the isolation of *Enterobacteriaceae* from *Rhipicephalus spp*. Both *E. coli* and *Salmonella spp* were isolated from this type of ticks with variation in the isolation rates this may be attributed to ecological factors.

Anterobacteriaceawere the most common genera of bacteria isolated from ticks. A previous study reported that ticks have a hematophagous habit, host body fluids, and attaching at predilection sites [22] However the same author isolated *Staph. aureus* and *Salmonella* from adult females ticks and their eggs.

The biological nature of the ticks and its blood feeding nature lead to increase the diversity of microorganisms in the ticks [23]. Moreover [24] isolated *Enterobacter*, *Pseudomonas*, and *Staphylococcus* from homogenates of *Rhipicephalus microplus* from the ticks.

A study performed by [2], who referred to microbiological techniques for the diagnosis of tick-borne pathogens. One hundred sixty-four bacteria were isolated from the ticks. These bacteria were as follows: 119 *E.coli*, 18, *Enerobacter aerogenes*, 11, *Salmonella spp*, 10, *Klebsiella spp* and 6 *Shigella spp*.

V. Conclusion

In conclusion, the results of this study approved the ability of hard ticks *Rhipicephalus spp.* to act as a vector for the Enterobacteriaceae The authors recommended further studies on epidemiological occurrence and the influence of environment, tick related risk factors, and tick-borne diseases, to provide a benchmark to design appropriate and control and prevention of ticks in the study area.

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