

ISOLATION AND IDENTIFICATION *Escherichia Coli* AND *Klebsiella Pneumonia* FROM TICKS *Hyalomma* SPP.KOCH, 1844FROM SHEEP IN BASRAH CITY

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ABSTRACT

The study was included isolation of ticks from ear, tail and udder of 60 sheep began from February to April (2012), The tick samples were identification and assign to type *Hyalomma* spp depended 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 has like tail inside at the end . The blood were taken from its and were growing on MacConkey and Eosin methylene blue agars, the bacterial colonies were growing in Eosine methylene blue agar was *Escherichia coli* which is appear as green metallic sheen, However the colonies on MacConkey agar was opaque, pink in color and mucus in natural which refer to bacteria *Klebsiella pneumonia*, The number and percentage positive of infection by these bacteria was 8(13%) for *Escherichia Coli* and 3(5%) for *Klebsiella pneumonia*.

INTRODUCTION

Tick are known as vector of various pathogenic agent that cause serious disease for human and domestic animals .All tick undergo four basic stages in their life cycle- Eggs , larva , nymph and adult. Furthermore, they have one host tick, like *Boophilus* spp, two host tick and three host tick like *Rhipicephalus* spp(6). Whether(17) to the mentioned most abundant ticks found in the ears, eyelids, lips of sheep and goat like *Hyalomma anatolicum anatolicum*, *Hyalomma marginatum sensu lato*, *Rhipicephalus haemophysaloides* and *Haemaphysalis bispinosa*. Tick bite might be causing directly mechanical tissue damage , irritation , hypersensitivity , abscess and when present in large number would cause anemia and reduce productivity (13); (20). Even though tick also could have transmitted diseases like Babesiosis, Theileriosis,

Anaplasmosis(14). In addition, Large *Babesia*spp.isolated from sheep and goat which was transmitted by *Rhipicephalus sanguineus* and *Hyalomma anatolicum anatolicum*(7). In turkey Cremean –Congo hemorrhagic fever (CCHFV) could infected both human and animals were transmitted by many types of Ixodes like *Haemophysalis concinna* , *Hyalomma anatolicum* , *Hyalomma detritum*, *Hyalomma marginatum* , *Rhipicephalus bursa* *Rhipicephalus turanicus*(16). while Tick –borne encephalitis virus (falvivirus) transmitted by ixodidae and Argasidae in Africa,Australia,America (5).

Also ticks consider as a potential vector for reservoir certain of infectious agent e.g *Pasteurallum multocida*, *Brucella abortus* and *Salmonella typhimurium* in man and animals (8). The hard tick *Rhipicephalus sanguineus* been vector for *Rickettsia conorii* (cause spotted fever disease) and *Coxiella burnetii* (cause Query (Q) fever) (3). While (4) refer that *Borrelia*spp were isolated from soft tick *Argas persicus* in Ethiopia .

(2) was remind the sheep were infestation by heavy ticks of *Hyalomma anatolicum anatolicum* and *H. asiaticum asiaticum* would cause mechanical damage and inflammation of interdigital lead to lameness.

The study aimed to identification tick types of sheep with try isolation bacteria from its.

MATERIAL AND METHOD

1- Sample collection (tick) :

-Tick samples were collected from ears , tail and udder of 60 illness sheep (male and female) from animals barns and veterinary house in north of basrah (Qurna city) between the period from February to April(2012) .The tick samples removing by forceps and laid in petri dish, select tick engorgement by blood (full with blood) and it had been punctured by needle and other by incision for blood swab then were spilt in sterile container have nutrient broth. The tick samples were kept in Test tub which contain ethyl alcohol 70 % and then transferred it to laboratory.

-Culturing : the broth samples incubated for 24 hours in 37 ° culture was done by use loop full from broth and streaked it in three agars MacConkey agar , nutrient agar and Eosin methylene blue agar plates were incubated in 37 ° for 24 hours.

- Uses Biochemical test : in this test were used citrate , Metthyle red , indol , ureas , TSI(H₂S) tests .

RESULT

A- Identification of Tick :All ticks would identified and assigned to *Hyalomma* spp according to the (19) , the following point would refer to diagnostic characters:

- 1- festoon present in male but un clear in female especially in engorgement some time none present in other species .
- 2- eyes present and other none .
- 3- pedipalps longer or short .
- 4- The spiracles plate like long comain male but triangular in female (internal end of spiracle have tail curved) .
- 5- female have scutum but male none .
- 6- male have adanal and subanal plate.
- 7- The legs in both sex were banded .(Fig .1-6) show *Hyalomma* spp.

B-Examination of colonies :

Identification of *Escherichia.coli* and *Klebsilla pneumoniait* according to (18).

1- The conventional biochemical test show *E.coli* positive in methyle red, indol but *Klebsilla pneumonia* positive in citrate and urease , (table. 1).

2-The colonies in Eosin methylene blue (EMB) agar were metallic sheen in appearance that refer to bacteria of *E.coli*(Fig.7,8), The colonies in MaCconkey agar were opaque , mucus and pink in color that refer to *Klebsilla pneumonia*(Fig. 9,10) .

The total number and percentage ratio was positive from 60 sheep samples are (8) Or (13%) of *E.coli*(table . 2)and 3 or (5%) were positive to *K . pneumonia*(table (3) .

Table (1)Biochemical test:

Type of bacteria	Citrate	Methyle red	indol	ureas	TSI(H ₂ S)
<i>E.coli</i>	—	+	+	—	—
<i>K. pneumonia</i>	+	—	—	—	+

Table (2) : Show number and percentage of positive samples of *E.coli* from ticks

Region	Samples number	Positive
Tail	20	3
Udder	20	4
Ear	20	1
Total	60	8
Percentage (%)		13 %

Table (3):Show the number and percentage of positive samples of *Klebsillapneumonia* from tick.

Region	Samples number	Positive
Tail	20	2
Udder	20	1
Ear	20	0
Total	60	3
Percentage (%)		5 %



Fig (1): *Hyalomma* spp. (female) with dorsal view isolated from tail of sheep X 40.



Fig (2): *Hyalomma* spp. (female) with ventral view isolated from tail of sheep X 40.



Fig (3): *Hyalomma* spp. (female) engorgement with dorsal view isolated from tail and ear of sheep X40.

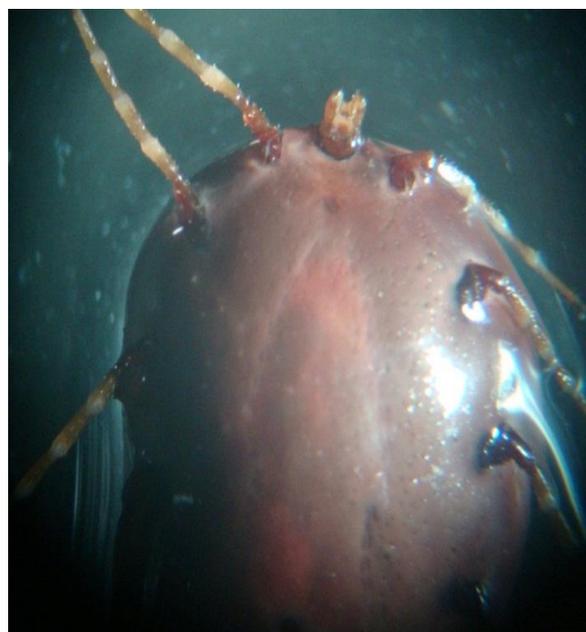


Fig (4): *Hyalomma* spp. (female) engorgement with ventral view isolated from tail and ear sheep X 40 .



Fig (5): *Hyalomma* spp. (male) with dorsal view isolated from ear of sheep X40.



Fig (6): *Hyalomma* spp. (male) with ventral view isolated from ear of sheep X 40.



Fig(7):*E.coli* show metallic sheen inEosin methylene blue (EMB) agar X 40.





DISCUSSION

In this study ticks had been isolated from 60 sheep with engorgement by blood or other full engorgement, Samples were collected at period between February to April (2012) and was more abundant in ear, tail and udder, it might be activation period to complete their life cycle or be growing. However, (11); (9) were reported the larvae and nymph of *Hyalomma* spp always stick on hairless area of ear, head and anal region of sheep and goats especially in early spring season.

Through the essential diagnostic characters of isolated ticks have been assigned to *Hyalomma* spp by according to (19) which was given differential diagnosis for all types of family of (Acari: ixodidae) further more (15) also referred the type of *Hyalomma* spp is more abundant in sheep and goat after collected 158 ticks and given identification for it. In addition ticks consider important as vector for many disease or pathogen. nevertheless, try isolation of both *E.coli* and *Klebsilla pneumonia* that actually confirm the *Hyalomma* spp could borne internally or in hemocoel one of important bacteria beside have borne protozoa or virus, this consequence agreement with (12) which was isolated GFP-expression *E.coli* from midgut of tick *Ornithodoros moubata* of sheep, in addition (21) either isolated bacteria of *Borrelia burgdorferi* from midgut of *Ixodes ricinus* through the grew it in BSK media.

In addition the isolation of this type of bacteria could prove the ticks carried off infectious and may cause secondary infection or respiratory or sometime intestinal inflammation beside what cause other disease.(1) were reported different isolation of bacteria from nasal cavity of lambs which was include *Corynebacterium Streptococcus* ,*E.Coli*, *Pseudomonas*, *Staphylococcus saprophyticus* and *Klebsiella pneumonia*. In conclusion the *Hyalommaspp* predominant type sheep especially in activation season bacteria which was *E.Coli* and *Klebseilla pneumonia* are important bacteria could cause infection and effect in health of animals .

عزل وتشخيص بكتريا *Escherichia Coli* و *Klebseilla pneumonia* من قراد
Hyalomma spp
Koch, 1844 من الاغنام في محافظة البصرة

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الخلاصة

تضمنت الدراسة عزل عينات قراد من اذن وذيل وضرع 60 من الاغنام بدأت من شهر شباط الى نيسان 2012 عرفت العينات على انها من نوع *Hyalomma spp* اعتمادا على الصفات التشخيصية وهي وجود النقوش او عدمه ، الارجل وكأنها مربوطة، أم اشكل الفتحات التنفسية فهي تشبه الضمة حيث طويلة في الذكر، ومثلثة الشكل في الاناث مع وجود ما يشبه الذيل في مؤخرتها . اخذت عينات دم من هذا النوع من القراد وتم تنميته على او ساط زرعيه وهي *Eosin methylene blue agar* *sagar* *MaCconkey*. المستعمرات البكتيرية التي نمت على وسط *Eosin methylene blue agar* كانت *Escherichia Coli* واتي ظهرت باللون الاخضر المعدني اليراق، اما المستعمرات على وسط *MaCconkey agar* تميزت باللون الورد المعتم وذات طبيعة مخاطية والتي اشارت بكتريا *Klebseilla pneumonia*. اما اعداد و نسب الاصابة الموجبة للقراد المصاب بهذه البكتريا فكانت 8 (13%) لبكتريا *Escherichia..coli* و3 (5%) لبكتريا *Klebseilla pneumonia* .

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