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 wereidentificationand assign to type*Hyalomma* sppdepended on diagnostic characters whichincluded: being festoons or none, legs appeared like banded andShape of spiracle like long coma in male,triangularshape in femalewith haslike tail inside at the end . Theblood were taken from its and were growing onMaConkeyand Eosin methylene blue agars,thebacterial colonies were growing inEosine methylene blue agarwas *Escherichia coli*which is appear as green metallic sheen, However thecoloniesonMaConkey agar wasopaque, pink in color and mucus in natural which refer tobacteria *Klebseilla pneumonia*, The number and percentage positiveof infectionby these bacteria was 8(13%) for*Escherichia Coli*and 3(5%) for *Klebseilla 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*sp , 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*Hyalommaanatolicumanatolicum, Hyalommamarginatumisaaci, Rhipicephalushaemophysaloides*and *Haemophysalisbispinosa*.Tick bit might be causingdirectly 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 havetransmitted diseases like Babesisios, Theileriosis,

Anaplasmosis(14). In addition, Large *Babesia*spp.isolated from sheep and goatwhich was transmitted by *Rhipicephalussanguineus*and *Hyalommaanatolicumanatolicum*(7).In turkeyCremean –Congo hemorrhagic fever (CCHFV) could infected both human and animals were transmitted by many types of Ixodeslike *Haemophysalisconcinna , Hyalommaanatolicum , Hyalommadettitum, Hyalomma marginatum , Rhipicephalus bursa Rhipicephalusturanicus*(16).while Tick –borne encephalitis virus (falvivirus) transmitted by ixodidae and Argasdae in Africa,Australia,America (5).

Also ticksconsider as a potential vector for reservoir certain of infectious agent e.g*Pasteurallamultocida*, *Brucellaabortus* and *Salmonella typhimurium* in man and animals (8).The hard tick *Rhipicephalus sanguineus*been vector for*Rickettsia conrii*(cause spotted fever disease) and *Coxiellaburnetii* (cause Query (Q) fever) (3). While (4) refer that *Borrelia*spp were isolated from soft tick *Argaspersicus*in Ethiopia .

(2)was remind thesheep were infestation by heavy ticks of *Hyalommaanatolicumanatolicum and H.asiaticumasiaticum*would cause mechanical damage and inflammation of interdigital lead to lameness.

The study aimedto identification tick types of sheepwith 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 toApril(2012) .The tick samples removing by forcepsand laid in petri dish, select tick engorgement by blood (full with blood) and it had been punctured by needle and other by incision forblood 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 pneumonia*it 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) 0r (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(H2S)
E.coli	—	+	+	—	—
K. pneumonia	+	—	—	—	+

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 sheepX 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.

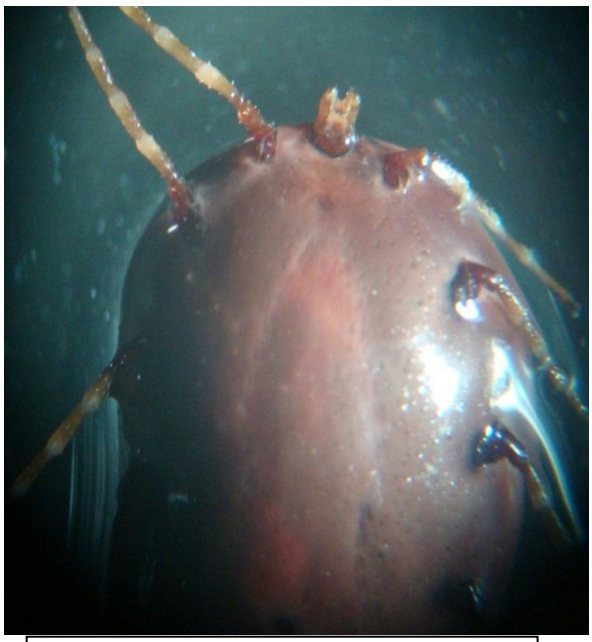

Fig (4): *Hyalomma* spp. (female) engorgement with ventral view isolated fromtail and ear sheep X 40 .

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

Fig (6): *Hyalomma s*pp. (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 toApril(2012)and was more abundant inear, tail and udder, it might be activationperiod tocomplete their life cycle or be growing. However,(11); (9)were reportedthe larvae and nymph of *Hyalomma* spp alwaysstick on hairless area of ear, head and anal region ofsheep and goatespecially in early spring season.

Through theessential diagnostic charactersof isolated ticks have been assign to*Hyalomma*sppby according to (19) which wasgiven differential diagnosis forall types of family of(Acari: ixodidae) further more(15)alsoreferred the type of *Hyalomma*sppis moreabundant in sheep and goat after collected 158 ticksand given identification for it. In addition ticks consider important as vector for many disease or pathogen. nevertheless,tryisolationof both*E.coli* and *Klebsilla pneumonia* that actually confirm the *Hyalomma*sppcould borne internally or in hemocoel one of important bacteria beside haveborne protozoa or virus, this consequence agreement with (12) which was isolated GFP-expression *E.coli* from midgut of tick *Orinithodoros moubata*of sheep,in addition **(**21) either isolated bacteria of *Borreliaburgdorferi*frommidgut of *ixodes ricinus*through the grew it in BSK media.

In addition the isolation of this type of bacteria could prove theticks 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 *Corynbacterium Streptococcus ,E.Coli, Pseudomonas, Staphylococcus saprophyticus and Klebsiella pneumonia*.

In conclusion the *Hyalomma*sppredominant 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 ,1844من الاغنام في محافظة البصرة

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الخلاصة

تضمنت الدراسة عزل عينات قراد من اذن وذيل وضرع 60 من الاغنام بدأت من شهرشباط الى نيسان 2012 عرفت العينات على انها من نوع *Hyalomma* spp اعتمادا على الصفات التشخيصية وهي وجود النقوش او عدمه ، الارجل وكأنهامربوطة، أم اشكل الفتحات التنفسية فهي تشبه الضمة حيثطويلة 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 MaCconkey agarو Eosin methylene blue agar.المستعمراتالبكتيرية التي نمت على وسطEosin methylene blue agarكانت*Escherichia Coli*واتي ظهرت باللون الاخضرالمعدني البراق، اما المستعمرات على وسط MaCconkeyagar تميزت باللون الوردي المعتم وذات طبيعة مخاطيةوالتي اشارت بكتريا *Klebseilla pneumonia* .اما اعداد و نسب الاصابة الموجبة للقراد المصابهذه البكتريا فكانت **8** (13%) لبكتريا *Escherichia..coli*و**3**(5%) لبكتريا*Klebseilla pneumonia* .

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