

A NOVEL BACTERIA RECOVERED FROM CASES OF SUBCLINICAL MASTITIS IN EWES

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ABSTRACT

Four hundred milk samples were collected from 205 local breed ewes for the detection of clinical and sub clinical mastitis. The incidence of clinical mastitis was (7.25%) while that of sub clinical mastitis was (26.25%). *Histophilus ovis* was isolated from natural cases of sub clinical mastitis of ewes and is a novel bacterium to Iraqi mastitis literature

INTRODUCTION

Mastitis means inflammation of the parenchyma of the mammary gland regardless of the cause, it causes up to 10% of all ewe deaths (1). It is one of the most costly diseases affecting the dairy industry, as it is responsible for decreased milk production, increased veterinary bills and treatment costs, higher labour costs and culling of affecting animals, in addition to altering the quality of the produced milk (2).

In a field investigation in Greece, clinical mastitis was recorded in 11.4% of examined ewes (3). In Iraq, mastitis is one of most important economical diseases to the animal welfare, the reported infection rates of clinical ovine mastitis ranged between 3.55%(4) to 8.6%(5). Roberts (6) was the first researcher isolating *Histophilus ovis* from a case of acute ovine mastitis in Australia, after that, it was isolated from a case of ovine mastitis in Wales. (7) Webb (8) and (1) considered *H. ovis* as an occasional causative agent of ovine mastitis.

This investigation aimed for detecting and isolating this bacterium from cases of natural clinical and subclinical mastitis for the first time in Iraq.

MATERIAL AND METHODS

Samples and data collection.

Milk samples were collected at Al-Qa'im district (about 375 km west of Baghdad) from 400 udder halves belonging to 205 local breed (Al-Nu'aymi breed) ewes from October 1998 to March 1999, the ewes aged between 2-9 years. Clinical mastitis was detected by the presence of abnormalities in udder or its secretion.

The teats were washed, dried and sterilized with iodine solution (diluted according to manufacturer's instructions) and the orifice was then scrubbed with ethanol 70%. From each half, about 20 ml. of mid stream milk were collected in two sterilized tubes (one tube was subjected to California Mastitis Test to investigate subclinical mastitis, while the other tube was frozen at -20°C for less than 5 days to be used for bacterial isolation later). Laboratory examination of milk samples

The samples were thawed at 37°C, mixed by shaking and then, for bacterial isolation, plated aerobically on 5% sheep Blood Agar (Oxoid), incubated at 37°C for 24-48 hours and Gram stain and biochemical identification were applied on purified bacterial culture according to (9) for all the bacteria isolated. For *H. ovis* after the biochemical identification the satellitism test was done to detect the requirement of this bacteria for X factors.

Antibiotic Sensitivity test

Antibiotic Sensitivity test were done according to (10). The following antibiotics were used: ampicillin (25 jig), amoxicillin (10 ug), bacitracin (10 IU), cephalixin (30 ug), chloramphenicol (30 jig), cloxacillin (5 jig), erythromycin (15 jig), penicillin-G (10 IU), tetracycline (30 jig), gentamicine (10 jig), rifampicin (5 jig) streptomycine (10 jig), nitrofurantion (300 jig), neomycine (30 jig) and trimethoprim - potentiated sulphamethoxazole (1.25 jig + 23.75 jig), the tests were carried out on the novel bacteria.

RESULTS

Rates of infection

Out of 400 samples examined bacteriologically, 122 (30.5%) samples were culturally positive for bacteria (24 samples were from clinical mastitis while 98 samples were from subclinical mastitis) including 3 cases of mixed bacterial infection.

The isolated bacteria

Staphylococcus Aureus, *Coagulase-Negative Staphylococci*,
Streptococcus Uberis, *Actinomyces Pyogenes*, *Pasteurella*

Haemolytical, *Histophilus ovis* were also isolated as shown in table (1)

Histophilus ovis was isolated from 2 cases of subclinical mastitis, one of them was a pure isolate while the another was mixed with *A.pyogenes*.

H. ovis isolates was characterized by the following:

The macroscopical and microscopical appearance and biochemicals of *H. ovis* are mentioned only, for the other previously isolated bacteria are not mentioned.

1- macroscopically:

*Did not grow on sheep blood agar.

^Grow on sheep blood agar cultured by *S.aureus* as white grayish minute colonies (positive satellitism test). "Grow aerobically and anaerobically on chocolate agar. "Grow on nutrient agar as white minute colonies.

2- microscopically: gram negative coccobacilli appears as pairs like diplococci, non-spore forming and non-inotile

3- biochemical tests:

* Requirement for X factor: +

* Requirement for V factor: -

*Catalase: - "Oxidase: +

*Hemolysis: -

*Mannitol: +

*Indole: -

Antibiotic Sensitivity test of *H. ovis*

Both isolates of *H. ovis* were sensitive to ampicillin, erythromycin, gentamicine, rifampicin, cephalixin, nitrofurantoin and neomycine. They were resistant to amoxicillin, bacutracin, penicillin-G, tetracycline, cloxacillin and trimethoprim- potentiated sulphamethoxazole. One isolate was sensitive to streptomycine and chloramphenicol.

DISCUSSION

The rates of clinical mastitis (7.51%) and the subclinical mastitis (26.25%) reported in this investigation are slightly higher than the rates previously reported in Iraq (11;5;4) which ranged between 3.55% to 8.6%, such differences could be easily attributed to the negligence of some microorganisms as causative agents of mastitis in their investigation and to the area of their studies.

The previous negligence of *H. ovis* by the above authors, all focus and high light the future importance of *H. ovis* as a possible and- poorly investigated cause which may lead to unestimated financial losses due to the clinical (6,7) and subclinical (this investigation) ovine mastitis caused by it. It appears that bacteria will continue to be the main accusers of losses in cases of ovine mastitis although losses could be attributed to other kinds of

microorganisms.

CONCLUSION

A new isolate of bacteria was recovered in this investigation, similar findings in other places will solidify this result, and experimental infection of ewes with the recovered new bacterial isolate will pinpoint its role as a causative agent of mastitis in ewes.

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The isolated bacteria	Bacteria isolated from cases of clinical mastitis (%)	Bacteria isolated from cases of subclinical mastitis (%)	The total sum
S.aureus	20 (83.33)	42 (42.86)	62 (50.81)
Coagulase-negative Staphylococci.	—	37 (37.76)	37 (30.33)
S.suberis	2 (8.33)	2 (2.04)	4 (3.28)
A.pyogenes	2 (8.33)	2 (2.04)	4 (3.28)
P.haemolytica	—	9 (9.18)	9 (7.38)
H.ovis	—	2 (2.04)	2 (1.64)

Table1; the isolated bacteria from cases of ovine mastitis

عزل بكتريا أصلية من حالات التهاب الضرع تحت السريري في النعاج

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الخلاصة

تم جمع ٤٠٠ عينة حليب من ٢٠٠ نعاج محلية للتحري عن التهاب الضرع السريري وتحت السريري. كانت نسبة الإصابة بالتهاب الضرع السريري (٧٠،٢٥%) ونسبة الإصابة بالتهاب الضرع تحت السريري (٢٦،٢٥%) وقد تم عزل بكتريا الهستوفيليس الضائية Histophilus ovis من حالات التهاب الضرع تحت السريري في النعاج وهي عزلة أصلية تعزل لأول مرة في العراق من حالات التهاب الضرع في النعاج.

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