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# SUBCLINICAL MASTITIS IN DAIRY EWES AT KAFR EL-SHEIKH GOVERNORATE,EGYPT AND OBSERVATION ON BACTERIA ASSOCIATED WITH IT

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## ABSTRACT

*Abacteriological survey for studying the prevalence and bacteria associated with subclinical mastitis was carried out in 10 sheep flocks at kafr El-sheikh Governorate .Atotal of 245 milk samples were collected aseptically from 202dairy ewes. The prevalence of subclinical mastitis was 24,1% in gland and 30,7%in dairy ewes .Coagulase negative strains (CNS) were the most prevalent bacteria representing 67.3% of the isolates ,Staphylococcus epidermidis 45.9%was the most prevalent species followed by Staphylococcus haemolyticus 7.1%, Staphylococcus xylosus 6.1%, Staphylococcus Simulans 6.1%, Staphylococcus Caprae 2%.*

*Corynebacterium to be the second place in importance and representing 11.2% of the isolates, also Staphylococcus aureus 7.1%, Escherichia coli 5.1%, Streptococcus agalactiae 3%, Pseudomonas aeroginosa 1%, Pasteurella haemolytica 1%.*

*The in vitro sensitivity pattern of the isolated organisms against 7 antibiotics was tested, Flumequine and Gentamycin gave the best results in inhibiting all the tested strains ,while Cloxacillin ,Neomycin and Oxytetracycline have moderate result but the most strains were resistant to penicillin .It is concluded that subclinical mastitis in dairy ewes is aserious problem in the surveyed area.*

## INTRODUCTION

Mastitis is inflammatory response of mammary gland to physiological and metabolic changes,trauma ,allergies,most frequently, caused by infection with pathogens ,injury and rarely by allergy and neoplasm Menzies and Ramanoon.2001.The importance of sub-clinical mastitis return to high prevalence rate ***Marco,(1994)*** and associated decrease in milk yield, growth retardation and high mortality rate among lambs in suckling ewes ***McCarthy et al.,(1988); Dario et al.,(1996);Peris et al.,(1996);***and ***saratsis et al.,(1999*****).** Many studies have defined prevalence of individual pathogens at one point of lactation. .Prevalence of intrammary infections (IMI) depend on the incidence of infection and the duration of existing infection**.** The infection manly either contagious (*Staphylococcus aureus ,Streptococcus agalactiae and streptococcus bovis*) or environmental (*Escherichia coli,Pseudomonas aeruginosa,Streptococcus uberis,and Staphylococcus chromogenes and other coagulase negative* strains *(CNS*) ***Bergonier et al.,(2003)***.Mastitis is highly maltifectorial and presents adifferent degrees of intensity, duration and consequences ***Benites et al.,(2000)***. This means that disease manifestations and the pathogen patterns are interrelated with apathogen of animal and environmental factors in acomplex fashion ***Vaarst and Enevoldsen,(1997).*** Litter, air, water, feces, improper milking practices and process of sucking are regarded as the main sources of mastitis pathogens ***Burriel,1998;Waage et al.,(1988).***

The aim of the present study was to determine the, bacteria associated with such infection and to determine the favorable antibiotic used in the treatment of subclinical mastitis.

# MATERIAL AND METHODS

## Animals:

The investigation was carried out on 202 native dairy ewes from 10 flocks in period from january to September .Ewes selected for this research were carefully examined to confirm the absence of signs of clinical mastitis, such as fever, pain or gland swelling and small quantity of milk was checked visually for signs of mastitis milk.

## Milk sampling:

Before milking, teats ends were carefully cleaned with cotton wool impregnated with 70% ethanol and previous discard of the first three stream and 10ml of milk as a samples were taken aseptically in asterile tubes .Samples were kept at 4c during transport to the laboratory for bacteriological analysis, which was carried out immediately withen 2 to 4h after collection.

## California Mastitis test (CMT):

All milk samples subjected to (CMT) according to ***Schalm et al., (1971)*** and the results were classified in four scores 0=negative,1=weak positive,2=distinct positive and 3=strong positive.

## Bacteriology:

From each milk samples (0.01ml)was spread evenly on 5% Sheep blood agar (bioMerieux S. A.) and MacConkey agar plates. The plate were incubated aerobically at 37c and examined after 24- 48h. According to ***Contreras et al.,(1997) Marco(1994),*** the presence of five or more bacterial colonies of the same type grew and with CMT positive, the

samples recorded positive ***Stefanakis et al., (1995)***. Absence of growth of fewer than five colonies consider negative. Growth of two different types of colonies with more than five colonies was defined as mixed infection .Growth of three or more bacterial type or more was defined as contaminated culture. Bacteria were identified microscopically ,morphologically and biochemically according to ***Cruickshank et al.,(1975).***

## RESULTS

In our study .245milk samples were collected from 202dairy ewes,116 (47.3%)milk samples and 90( 44.6% ) of examined dairy ewes were positive. Using subclinical mastitis (SCM) for the demonstration of both bacteriologically positive and California mastitis test (CMT) positive, 62(30.7%) of the examined ewes and 59 (24.1%) of the examined gland were affected (table: 1 and 2).

**Table (1):** Results of CMT and bacterial culture on the examined ewes(202).

| | **Bacteriology** | | | | **Total** | |
|---|---|---|---|---|---|---|
| | **+(positive)** | | **- (negative)** | | | |
| **CMT** | **No** | **%** | **No** | **%** | **No** | **%** |
| CMT+ve | 62 | 65.9 | 28 | 25.9 | 90 | 44.6 |
| CMT-ve | 32 | 28.6 | 80 | 74.0 | 112 | 55.4 |
| **Total** | **94** | **46.5** | **108** | **53.5** | **202** | **100** |

**Table (2):** Results of CMT and bacterial culture on the examined milk samples(245).

| | **Bacteriology** | | | | **Total** | |
|---|---|---|---|---|---|---|
| | **+(positive)** | | **- (negative)** | | | |
| **CMT** | **No** | **%** | **No** | **%** | **No** | **%** |
| CMT+ve | 59 | 60.2 | 57 | 38.8 | 116 | 47.3 |
| CMT-ve | 39 | 39.8 | 90 | 61.2 | 129 | 52.7 |
| **Total** | **98** | **40** | **147** | **60** | **245** | **100** |

Of the 98 collected milk samples from glands with subclinical mastitis 94 (95.9%) yield bacteria in pure culture. Cagulase negative strains *(CNS)*, were the predominant organisms (67.3%) followed by *Corynebacterium* (11.2%), *Staphylococcus. Aureus* (7.1%), *Escherichia coli* (5.1%) and other bacteria .And 4(4.0%) samples yield mixed culture (Table 3).

**Table (3):** Incidence of bacterial isolates isolated from milk samples demonest-erated positive CMT score.

| No of examined samples | Type of organism | CMT poitive culture | |
|---|---|---|---|
| | | **No** | **%** |
| | **Single infection** | | |
| | ***CNS:*** | 66 | 67.3 |
| | *- S .epidermidis* | 45 | 45.9 |
| | *- S.haemolyticus* | 7 | 7.1 |
| | *- S.xylosus* | 6 | 6.1 |
| | *- S.simulans* | 6 | 6.1 |
| | *- S.caprae* | 2 | 2.0 |
| | ***Corynebacterium:*** | 11 | 11.2 |
| | *- C.pseudotuberculoses* | 6 | 6.1 |
| | *- C.bovis* | 5 | 5.1 |
| | *- S aureus* | 7 | 7.1 |
| | *- E.coli* | 5 | 5.1 |
| | *- S.agalactea* | 3 | 3.0 |
| | *- Ps.aerogenosa* | 1 | 1.0 |
| | *- P.haemolytica* | 1 | 1.0 |
| | **Total** | **94** | **95.9** |
| | **Mixed infection** *Saureus+E.coli* | 2 | 2.0 |
| | *- CNS+corynebacterium* | 1 | 1.0 |
| | *- Strept.agalactiae+P.haemoltica* | 1 | 1.0 |
| | **Total** | **4** | **4.1** |

| | | | |
|---|---|---|---|
| *S* | *Staphylococcus* | *P.* | *Pasteurella* |
| *C* | *Corynebacterium* | *CNS* | *Coagulase negative strains* |
| *E* | *Escherichia* | *Ps* | *PSeudomonas* |
| *S* | *Streptococcu.* | | |

**Table (4):** Antibiotic sensitivity test of the isolated strains from milk samples obtained from positive CMT.

| Type of micro-organism | No | A 20Mg | | F 30Mg | | C 5Mg | | G 10Mg | | O 30Mg | | N 10Mg | | P IU | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NO | % | No | % | NO | % | No | % | NO | % | No | % | No | % |
| *Staph.epidermidis* | 45 | 5 | 11.1 | 42 | 93.3 | 22 | 48.9 | 44 | 97.8 | 12 | 26.7 | 30 | 66.7 | 5 | 11.1 |
| *Corynebacterium S aureus* | 11 | 3 | 27.3 | 9 | 81.8 | 7 | 63.6 | 10 | 90.9 | 4 | 36.4 | 3 | 27.3 | 1 | 9.0 |
| *E.coli* | 7 | 4 | 57.1 | 6 | 85.7 | 5 | 71.4 | 6 | 85.7 | 5 | 71.4 | 3 | 42.9 | 1 | 71.4 |
| *S.agalactae* | 5 | 1 | 20 | 4 | 80 | 3 | 60 | 5 | 100 | 1 | 20 | 2 | 40 | 0 | 0 |
| *Ps.aeruginosa* | 3 | 0 | 0 | 3 | 100 | 2 | 66.7 | 2 | 66.7 | 0 | 0 | 1 | 100 | 0 | 0 |
| *P.haemolytica* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 100 | 0 | 0 | 1 | 100 | 0 | 0 |
| | 1 | 0 | 0 | 1 | 100 | 0 | 0 | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |

A.(Amoxcillin G.(Gentamycin
P.(Penicillin )
F.(Flumiquine) O.(oxytetracyclin )
N.(Neomycin)
C.(Cloxacillin )

## DISCUSSION

Many studies carried to estimate the prevalence of subclinical mastitis in milking sheep in different geographical areas ,the prevalence of subclinical mastitis in dairy ewes recorded in our study ,with 30.7% of dairy sheep.Similar result have been reported by ***Stefanakis et al.,(1995)*** and ***Las Heras et al.,(1999)*** who recorded prevalence rates of 30% and 30.6% respectively.Higher prevalence rates have been recorded by ***Winkler*** and ***Gootwine(1989)*** and ***Quiroga et al.,(1997)*** who mentioned the subclinical mastitis was 55% and 92% respectively in dairy ewes. In contrast ***AL-Majali and Jawabreh (2003)*** reported 18.3%.

Regarding to glands ,the subclinical mastitis was 24.1%. Higher prevalence was recorded by ***Ariznabarreta et al.,(2002)*** and ***Batavani et al.,(2003)*** who reported prevalence rates ranged from 39%-41%.While ***Al-Majali and Jawabreh(2003)*** recorded lower prevalence rates(10.4%).

The most frequently organisms isolated from culture positive samples was *Coagulase negative strains(CNS)*representing a percentage of 67.3%. Higher result recorded by ***Menzies and Ramanoon,(2001) ,Vieira-da-Motto et al.,(2001).,McDougall et al.,(2002), Moawad and Osman (2005).,*** *Staphylococcus epidermidis* is the most prevalent and wide distributed species of representing 45.9%, other species of *CNS,* of low importance and also widely distributed such as *Staphylococcus haemolyticus* 7.1%, *Staphylococcus xylosus*, *Staphylococcus simulans* 6.1%, *Staphylococcus caprae* 2%, were these results, with slight differences in the frequency of isolation for each species nearly similar to those found by ***De la Cruz et al.,(1994)***. *Cagulase negative strains (CNS)* pathogen have been considered to be the major cause of non clinical intramammary infectious ***Bor et al., (1989), Keisler et al., (1992).*** Also, ***Fthenakis (1988).***reported *CNS*. in 32% of teat canals and in 17% of teat sinuses of ewes in Britain in the absence of infection of the parenchyma.

*Corynebacterium* representing 11.2% of the isolate(*Corynebacterium Pseudotuberclosis* 6.1%, *Corynebacterium bovis* 5.1%),was the second bacterial pathogen in importance ,our result similar to those recorded by ***Ariznabarreta et al.,(2002)*** and ***Las Heras et al.,(1999).***While ***Watson et al., (1990)*** and ***Marco, (1994)*** isolated *Corynebacterium* in percentage 5%, 2% respectively.

*Staphylococcus aureus* is the 3rd.bacterial pathogen cause intramammary infection, isolated in apercentage of 7.1%from California mastitis test (CMT) positive samples ,this percentage mush lower than isolated by ***Moawad and Osman,(2005).,*** The high persistence of mastitis due to production of Exo-polysaccharide(Slime)which form aprotective barriers ***Baselga et al.,(1994).***

*Escherichia coli* and *Streptococcus agalactiae* were detected in apercentage 5.1% and 3% respectively. Our result nearly similar to that recorded by ***Maisi et al.(1987)*** and ***Lafi et al.(1994).***

Other bacteria were isolated in low percentage such as *Pseudomonas aerogenosa* and *Pasteurella haemolytcu*s (1% and 1%) these percentage agree with ***Albenzio et al.(2002)***.

Regarding to mixed infection, the most isolated bacteria were *Staphaphylococcus aureus* with *Escherichia coli* in percentage 2%, followed by *CNS* with *Corynebacterium* in percentage 1% and *Streptylococcus agalactae* with *Pasturella .haemolytica* in percentage-1%. The same bacteria isolated in mixed infection by ***Watkins et al.,(1991).***

Regarding the sensitivity of the isolated micro-organism to some antibiotic ,using 7differnt antibiotic to 7 isolated strains.

Most of all the isolated strains were sensitive to Flumequine, Cloxacillin and Gentamycin.Similarly the higher sensitivity of all strains of *Escherichia coli* to Gentamycin had earlier been reported by ***Ngeleka, et al., (1998).*** While 11.1% *Staphylococcus epidermidis*, 27.3% *Corynebacterium* 57.1% of the *Staphylococcus aureus* isolates and 20% of the *Escherichia coli* isolates appear to be sensitive to Amoxcillin.The prevalence of *Staphylococcus epidermidis*, *Corynebacterium*, *Staphylococcus aureus* and *Escherichia coli* isolates to Oxytetracycline were 26.7%,36.4%,71.4%,and 20% respectively.On the other hand, 66.7% *Staphylococcus epidermidis*,,27.3% *Corynebacterium*, 42%, *Staphylococcus aureus*, 40% *Escherichia coli*,100%, *Streptococcus agalactae* and 100%,*Pseudomonas .aeroginosa* isolates were sensitive to Neomycin This results slightly different with ***Rahman*** and ***Baxi (1983)*** and ***Kumar (1988).***

While all *Strept.agalactae ,Ps.auroginosa* and *P.haemolytica* were resistant to Amoxicillin Oxytetracycline and penicillin. The superior effect of Flumequin and Gentamycin than other antibiotics might be due to the fact that Flumequin seldom used as treatment of mastitis in contrast to other antibiotics which most frequently used and may leads to the development of resistant strains.

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## التهاب الضرع الكامن في الأغنام في محافظة كفر الشيخ - مصر وملاحظة البكتريا المصاحبة له

**يس رمضان عبد المولى عزب**

تم عمل مسح بكتريولوجي لمعرفة مدى انتشار البكتريا المصاحبة لإلتهاب الضرع الكامن في 10من قطعان الأغنام في محافظة كفر الشيخ .تم فحص 245عينة لبن من 202غنمة حلوب باستخدام اختبار شالم وكذلك الفحص البكتريولوجي,فكان مدي انتشار التهاب الضرع الكامن بنسبة 24% في الضرع وبنسبة 30.7%في الأغنام .ووجد ان الميكروب العنقودي السالب لاختبار الكواجيولاز اكثر الميكروبات المعزولة حيث تم عزلة بنسبة 67.3% وكان الميكروب العنقودي الجلدي

اكثر هذه الميكروبات تواجد حيث تم عذله بنسبة 45.9%. كما يليه العنقودى هيمولايتكم 7.1% ,الغنقودي زيلوزز 6.1% ,العنقودي سميولانس 6.1% ,والعنقودي كابري 2%.

يأتي ميكروب الكوريني باكتريم في الدرجة الثانية من حيث ألأهمية ويمثل 11.2% يليه الميكروب العنقودي الذهبي 7.1%وميكروب ألإشرشيا كولآي5.1% والميكروب السبحي ألأجالاكتيا 3% والسيدوموناس إرجنوزا 1% والباستيريلا هيمولتيكم بنسبة1%.

كما أجرى اختبار الحساسية لعدد 7من المضادات الحيوية لبعض العترات المعزولة, ووجد أن اكثر المضادات الحيوية تأثيراً هي الجينتاميسين والفلومكوين وأوسطهم تأثيراً الكلوكساسيلن, النيوميسين, الآوسيتتراسيكلين. واقلهم تأثيرا هو البنسلين.