BACTERIOLOGICAL STUDIES ON SUBCLINICAL MASTITIS OF SMALL RUMINANTS IN ASSIUT GOVERNORATE

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Abstract

A bacteriological survey studying the prevalence and etiology of subclinical mastitis in small ruminants was carried out on 113 dairy ewes in two groups differed in hygienic and feeding conditions (group I: 60 ewes belonged to a large governmental farm and group II: 53 ewes belonged to small private flocks) and group III: 50 she-goat belonged to small private flocks. A total of 313 milk samples were collected from udder halves (118, 101 and 94 milk samples from group I, II and III, respectively). Those which were classified by California Mastitis Test and bacterial culture as positive were considered to have glands with subclinical mastitis. The prevalence of subclinical mastitis was 5.08%, 16.83% and 37.23% in group I, group II and group III, respectively, showing highly significant increase ($P > 0.001$) in groups II and III than in group I. Bacteriologically:the most frequently isolates from subclinical mastitis milk samples were coagulase negative staphylococci (48.15% and 41.86%) followed by E. coli (18.52% and 30.23%), Staph. aureus (22.22% and 9.30%), and Strept. spp.(7.41% and 13.95%) from ewes and she-goat, respectively. In vitro antimicrobial susceptibility test of the most isolated strains revealed that the most effective antibacterial agents were Ofloxacin, Ciprofloxacin, followed by Gentamicin and Oxytetracycline with susceptibility 100%, 98.28%,84.48% and 84.48%, respectively. While the most tested strains resisted Penicillin, Cefotaxime and Ampicilin. It can be concluded that:

1. Prevalence of subclinical mastitis in she-goat was much more than in dairy ewes.
2. Staphylococcus spp. especially coagulase negative staphylococci were the most prevalentpathogens causing subclinical mastitis in small ruminants.
3. The bacterial isolates from cases of subclinical mastitis in small ruminants were sensitive tosome antibacterial agents.
4. The prevalence of subclinical mastitis in small ruminants related to hygienic measurements, management and feeding conditions.

INTRODUCTION

Mastitis is one of the most serious health and economic problems in sheep and goat flocks which have many adverse implications represented by decrease in quantity and quality of milk components, growth retardation of lambs and kids and pre-weaning mortalities (Jones and Watkins, 2000). Although ovine clinical mastitis is typically gangrenous and causes death, the important economically is subclinical mastitis due to its prevalence rate (Marco, 1994). The subclinical inflammations of the

mammary gland cause oxidation stress because the generation of free radicals is much higher than that of antioxidant (Jóźwik et al., 2010).

The udder infection originates either from haematogenous, lymphogenous, cutaneous or galactogenous routes and influenced by different environmental factors including feeding, housing, bedding and management (Menzies and Ramanoon, 2001).

Diagnosis of subclinical mastitis is commonly based on cytological examination as well as biochemical changes in milk but conformation must be based on bacteriological examination (Radostitis et al., 2000).

Intramammary infections in dairy small ruminants are mainly of bacterial origin by either contagious pathogens (Staphylococcus aureus and Streptococcus agalactiae) or environmental pathogens (E. coli, Pseudomonas aeruginosa, Strept. uberis and coagulase-negative Staphylococcus sp.), (Radostitis et al., 2000 and Bergonier et al., 2003).Members of the genus Staphylococcus are the main etiological agents involved in all forms of mastitis in goats and sheep. Although Staphylococcus aureus has been considered the major pathogen, coagulase-negative staphylococci (CNS) are the most commonly observed, principally in sub-clinical mastitis (Batavani et al., 2003).

The objective of this investigation is to elucidate the prevalence, etiology of subclinical mastitis in sheep and goats in Assiut Governorate, as well as the antibiogram of the most isolated strains.

Keywords: subclinical mastitis, Sheep, goat, bacteriological examination.

MATERIALS AND METHODS

Animals:-

In this study, 163 dairy ewes and she-goats were used to study some aspects concerning subclinical mastitis recording their hygienic measurements and feeding status. The animals selected for this investigation were apparently healthy, free from any signs of clinical mastitis and other palpable udder lesions. The animals were divided into three groups.

Group I:-

Sixty dairy ewes reared in a large governmental farm, receiving good feeding& management and under good hygienic conditions. They were grazing on a green fodder, feed on concentrates and hay with vitamins supplement and fresh water adlib.

Group II:-

Fifty three dairy ewes reared in small private flocks.

Group III:-

Fifty dairy she-goats reared also in small private flocks.

The animals in both groups II and III suffered from poor management, poor hygienic measurements and bad feeding conditions as they were grazing on therubbish and roughages in streets and drinking water from stagnant places.

Sampling:-

Out of 326 mammary glands a total of 313 milk samples were collected aseptically from apparently healthy functioning gland (118 samples from group I, 101 samples from group II and 94 samples from group III), according to the method described by Al-Majali and Jawabreh (2003). Milk samples were collected while the ewes and she-goats were restrained in standing position, and the teat end of each half udder was scrubbed thoroughly using cotton soaked in 70% ethyl alcohol. The first three streams were discarded before 5 ml of the milk were collected in sterile containers. The samples were transported to laboratory on ice and tested within 6 h. of collection.

California Mastitis Test (CMT):-

It was carried out for all milk samples using Delaval Mastitis Test, 3804101, Poland, according to method described by Schalm *et al.* (1971).

Bacteriological Examination:-

All California Mastitis Test positive samples were thoroughly shacked and a standard loopfull (0.01 ml) from each milk sample was inoculated onto the surface of 5% sheep blood agar (Bacto-Agar, Diffco Laboratory), Mannitol salt agar (BBL), Azid blood agar plate and MacConkey agar (Biomark Lab. India). The inoculated plates were incubated overnight aerobically at 37°c. Modified EC-medium (Diffco No.7197405) with Novobiocin 2% tubes inoculated with a loopfull of each milk sample and incubated at 37°c for 24 hours, from these incubated tubes, a loopfull was streaked onto the surface of Sorbitol MacConkey agar plates (Diffco.). All plates were incubated aerobically at 37°C for 48h.

The presence of six or more bacterial colonies of the same type on the medium was considered to be significant and the sample was recorded as bacteriologically positive (Batavani *et al.,* 2003).The suspected colonies were identified: morphologically and biochemically confirmed according to Quinn *et al.* (1994).

Basis of clarifying subclinical mastitis:-

Mammary glands, without clinical abnormalities and with apparently normal milk, that both California Mastitis Test and bacteriologically positive were considered to have subclinical mastitis (Moawad and Osman, 2005).

Antibiogram:-

Antibiogram ofthe recovered isolates was adopted using antimicrobial susceptibility testing by disc diffusion standard technique according to Quinn *etal.* (1994). The isolated strains were tested against eight antibiotics (Ampicilin 10 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Neomycin 30 µg, Ofloxacin 5 µg, Oxytetracycline 30 µg and Penicillin 10 µ), (Bioanalyse-Turkey).

Statistical data analysis was done using Chi-square by SPSS, 2005 program (Statistical Package for Social Sciences for Windows Release 14.0.0.).

RESULTS

Detailed obtained results were illustrated in Tables (1-3).

Table 1.California Mastitis Test (CMT) and bacteriological examination of the examined milk samples.

Animals	CMT			Bacteriological examination			
				+ ve		- ve	
	result	No.	%	No.	%	No.	%
Group I	+ ve	8	6.78	6	5	2	1.7
No. of milk samples = 118	- ve	110	93.22	0	0	110	93.2
Group II	+ ve	20	19.80	17	16.8	3	2.9
No. of milk samples = 101	- ve	81	80.20	0	0	81	80.2
Group III	+ ve	43	45.74	35	37.2	8	8.51
No. of milk samples =94	- ve	51	54.26	0	0	51	54.3

**Highly significant statistical variations $\chi^2 = 7.989$ $p > 0.001$

Table 2. Incidence and Frequencyof the isolated bacteria causing subclinical mastitis insmall ruminantsfrom the examined milk samples.

Bacterial isolates species	Dairy ewes							She-goat (Group III)		
	Group I		Group II		Total					
	Incidence (n. = 118)		Incidence (n. = 101)		Incidence (n. = 219)		Frequency (n. = 27)	Incidence (n. = 94)		Frequency (n. = 43)
	No.	%	No.	%	No.	%	%	No.	%	%
Coagulase-negative staphylococcus	4	3.39	9	8.91	13	5.94	48.15	18	19.15	41.86
Staph. aureus	2	1.69	4	3.96	6	2.74	22.22	4	4.26	9.30
E. coli	1	0.85	4	3.96	5	2.28	18.52	13	13.83	30.23
Strept. spp.	0	0	2	1.98	2	0.91	7.41	6	6.38	13.95
Corynebact. spp.	0	0	1	0.99	1	0.46	3.70	0	0	0
Enterobacter spp.	0	0	0	0	0	0	0	2	2.13	4.65
Total	7	5.93	20	19.8	27	12.33	100	43	45.75	100

Staph. spp. were 70.37% and 51.16% in ewe and she-goat, respectively.

Table 3. In Vitro susceptibility pattern of the most frequent isolates against different antibiotics.

Isolated bacterial species / Antibiotic	Coagulase-negative staphylococcus (n. = 30)	*Staph. aureus* (n. = 10)	*E. coli* (n. = 18)
Ampicilin (10µg)	12/30 (40%)	4/10 (40%)	5/18 (27.8%)
Cefotaxime (3µg)	0/30 (0%)	0/10 (0%)	10/18 (55.6%)
Ciprofloxacin (5µg)	29/30 (96.7%)	10/10 (100%)	18/18 (100%)
Gentamicin (10µg)	23/30 (76.7%)	9/10 (90%)	17/18 (94.4%)
Neomycin (30µg)	20/30 (66.7%)	8/10 (80%)	11/18 (61.1%)
Ofloxacin (5µg)	30/30 (100%)	10/10 (100%)	18/18 (100%)
Oxytetracycline (30µg)	26/30 (86.7%)	9/10 (90%)	14/18 (77.8%)
Penicillin (10 µ)	3/30 (10%)	2/10 (20%)	1/18 (5.6%)

DISCUSSION

In recent years due to the high cost of large dairy cattle, milk of goat and sheep is used as human food. The udder health condition is therefore, of great importance for public health and economic importance. Subclinical mastitis is the most serious form as both infected udders and milk show no obvious clinical abnormalities, whereas several causative organisms are discharged with milk for long time (Salem *et al.,* 1993).

On the basis of clarifying subclinical mastitis as the presence positive results of both California Mastitis Test and bacterial isolation and through the present study, 6 (5.08%), 17 (16.83%) and 35 (37.23%) milk samples (glands) were affected with subclinical mastitis in groups I, II and III, respectively, as shown in Table (1). The prevalence of subclinical mastitis in regard to the gland in ewes through many studies varied widely as 4.5, 8.08, 9.1, 10.14, 26.2, 31.36, and 41% (Las Heras *et al.,* 1999, Sadek, 2008, McDougall *et al.* 2002, Al-Majali and Jawabreh 2003, Pengov, 2001, Moawad and Osman, 2005, & Batavani *et al.,* 2003, respectively).While the previously recorded subclinical mastitis prevalence in she-goats ranged as 15.23%, 16.9%, 18%, 25.5% and 40.2% (Sadek 2008, Sānchez *et al.* 2002, Contreras *et al.* 1995, McDougall *et al.* 2002 and Moroni *et al.* 2005, respectively).

Through the present work, the ewes in group II and she-goats in group III showed highly significant increase ($p > 0.001$) in prevalence of subclinical mastitis than ewes in group I, the latter which received a good management and good feeding. Animals in both groups II & III suffered from poor management, poor hygienic conditions and bad feeding as they were in nomadic rearing system but grazing on the rubbish and roughages in streets. The differences in the management, nutrition, size of flock, breed, and parity of the dam, lactation period, season, case definition and the diagnostic criteria used are the main factors the prevalence of ovine subclinical mastitis (Batavani *et al.* 2003). Moreover, other risk factor since she-goats (group III) were lactating all over the year without drying periods where the potential for ongoing transmission of infection from late to early lactation goats in the barns always present (McDugall *et al.,* 2002). Also the udder infection originates either from haematogenous, lymphogenous, cutaneous or galactogenous routes and influenced by different environmental factors including feeding, housing, bedding and management (Menzies and Ramanoon, 2001).

The present work cleared that she-goat, group III, were more affected with subclinical mastitis (Table 1), these results may be due to poor management, poor hygienic conditions and bad feeding. The difference in management between ewes

and goat and the housing of the goats may have been more heavily contaminated than that of the sheep due to continual use of the facilities. Additionally, as there were does lactating at all times of the year, the potential for ongoing transmission of infection from late to early lactation goats in the barns always present (McDugall *et al.,* 2002).

California Mastitis Test showed higher prevalence rate of subclinical mastitis than bacteriological examination (Table 1), since it has been standardized for cow's milk and it is most accurate in this species (Batavani *et al.*, 2003), however it is useful as screening test in ovine species but false positive results may be obtained due to the presence of non infectious factors (Al-Majali and Jawabreh, 2003, Moawad and Osman, 2005 & Lafi, 2006).In addition, ewe's milk tends to have a higher cell count, nuclear fragments, cytoplasmic particles and fat content in normal milk (Donovan *et al.,* 1992).

In the present investigation, the most frequent isolate from subclinical cases of small ruminants was coagulase negative staphylococci (48.15% and 41.86% in ewes and she-goat, respectively- Table 2). They have been considered as the major cause of subclinical mastitis in small ruminants in previous investigations (Menzies and Ramanoon, 2001, Pengov, 2001, McDougall *et al.,* 2002, Batavani *et al.*, 2003, Bergonier *et al.,* 2003, Da Silva *et al.,* 2004, Moawad and Osman, 2005, Moroni *et al.,* 2005, Spanu *et al.,* 2011 and Bagnicka *et al.,* 2011). On the other handthe prevalent bacterial species isolated from mammary glands of ewe with subclinical mastitis were *Staph. aureus* (39%), while coagulase negative staphylococci were 17.9% (Radostitis *et al.,* 2000, Al-Majali and Jawabreh, 2003). Also Sadek (2008) found that *Staph. aureus* was the highest pathogen frequency isolated from she-goat's milk samples with subclinical mastitis (33.33%) while coagulase negative staphylococci (30.77%).

The highest infection rate by coagulase negative staphylococci may be explained by the fact that coagulase negative staphylococci are encountered in the environment as an environmental pathogen. So it is able to colonizes the skin of animal, introduce from the skin to the gland by the process of suckling or during milking via teat canal and if they pathogenic may cause subclinical mastitis (Batavani *et al.*, 2003).

The incidence of the isolated coagulase negative staphylococci as environmental pathogens in groupsII of ewes and group IIIof she-goat(8.91% and 19.15%, respectively) was much more than in group I of ewes (3.39%) from milk samples of the affected glands (Table 2). The increasing in the environmental and opportunistic pathogens is mainly related to poor conditions of environmental hygiene

and/ or decrease defense of the mammary gland (Moroni and Cuccuru 2001 & Albenzio *et al.,* 2002).

Staph. aureus was isolated in a frequency percentage 22.22% of the isolated bacteria from mastitic ewe's milk samples(Table 2). Similar results were recorded by Batavani *et al.*(2003), Moawad and Osman (2005)and Fotou *et al.*(2011),(22%, 24.36% and24%, respectively),while higher ones were detected as 39% and 37% (Al-Majali and Jawabreh, 2003 and Da Silva *et al.,* 2004, respectively), and lower frequency as 2% and 7.1% (Pengov, 2001 andMcDougall *et al.,* 2002).In the present work, *S. aureus* showed 9.3 % frequency form she-goat's milk. Similar result was obtained by McDougall *et al.,* 2002 (10%), while relatively lower percentage was obtained by Moroni *et al.,* 2005 (6%).

Not only, because of the thermostable enterotoxins or the leukotoxins and other virulence factors, such as haemolysins and toxic-shock syndrome toxin produced by *Staph. aureus* (Taponen and Pyörälä, 2009) but also the organism has capacity to produce exo-polysaccharides "slime", forming a protective barrier that restricts the efficiency of the immune responses limiting the action of drugs (Contreras *et al.,* 2000) resulting in pathogen persisting into the gland. Since infected animals may represent a source of infection among the herd and it is of the most important to identify those animals harboring *S. aureus* (Da Silva *et al.,* 2004).

Other isolated bacteria were *E. coli* (18.52% and 30.23%, in ewe and she-goat, respectively), *Strept. Spp.* (7.41% and 13.95% in ewe and she-goat, respectively), *Corynebact. spp.* (3.70% in ewe) and *Enterobacter* spp. (4.65% in she-goat), as shown in Table 2. A high incidence of subclinical in she-goat due to *E. coli* may be due to fecal contamination, since Coliform organisms are parts of the environmental flora (Menzies and Ramanoon, 2001 and Faten *et al.,* 2005).*E. coli* was isolated in a previous studies by Al-Majali and Jawabreh, 2003 (17.9% in ewe), Moawad and Osman, 2005 (14.1% in ewe), Fotou *et al.*, 2011 (5% in ewe), Pengov, 2001 (1%) Faten *et al.,* 2005 (15% in she-goat) and Sadek, 2008 (4% and 5.13%, in ewe and she-goat, respectively). Also some authors isolated the microorganisms with different frequent percentage as *Strept. Spp.* by Pengov (2001), Al-Majali and Jawabreh (2003), Moawad and Osman (2005), Batavani *et al.* (2003), (3.8%, 25%, 7.69% and 4%, respectively in ewe), McDougall *et al.,* 2002 (2-6% in she-goat). *Corynebacteriumspp.* was isolated by Contreras *et al.*(1997) in she- goat with a percentage 8%. While Sadek (2008) isolated *Enterobacter spp.* from subclinical mastitic she-goats and ewe's milk samples with a high frequent percentage 20.51% and 12%, respectively.

Identification of the causative organism and sensitivity testing besides treatment or culling of untreatable animals are very important for control of sub-clinical mastitis, as the infected animals with subclinical mastitis act as primary source of infection to anther healthy one. In the present study, the prevalent isolated bacteria were tested for antibacterial sensitivity pattern as shown in Table (3). The obtained results revealed that the most effective antibacterial agents all over the study were Ofloxacin and Ciprofloxacin, with susceptibilityof 100% and 98.28%, respectively followed by Gentamicin and Oxytetracycline with susceptibility of 84.48% for both. On the other hand all tested strains resisted Penicillin, Cefotaxime and Ampicilin. Similar results were obtained by Mishra *et al.* (1996). Penicillin G was drug that demonstrated the highest *invitro* resistance rates when tested against both coagulase negative staphylococci and *Staph. aureus* (Da Silva *et al.,* 2004). Resistance of coagulase negative staphylococci and *S. aureus* isolates to penicillin G was also described by Lima Júnior *et al.* (1993) and Corrales *et al.* (1995). The resistance to Penicillin, Cefotaxime and Ampicilin observed in this study may be of concern since these drugs represents the main antibiotic group recommended for staphylococcal mastitis treatment. From the obtained results it can be concluded that:

1. Prevalence of subclinical mastitis in she-goat was much more than in ewes.
2. *Staphylococcusspp.* especially coagulase negative staphylococci were the most prevalent pathogens causing subclinical mastitis in small ruminants.
3. The bacterial isolates from cases of subclinical mastitis in small ruminants were sensitive to some antibacterial agents.
4. The prevalence of subclinical mastitis in small ruminants related to hygienic measurement, management and feeding conditions.

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دراسات بكتريولوجية علي التهاب الضرع الخفي في المجترات الصغيرة بمحافظة أســـــيوط

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أجريت هذه الدراسة لمعرفة مدي انتشار والمسبب البكتيري لالتهاب الضرع الخفي في المجترات الصغيرة. تم أجراء البحث علي ١١٣ أنثي غنم حلابة في مجموعتين (المجموعة الأولي عبارة عن ٦٠ أنثي غنم تربي في مزرعة حكومية كبيرة وتلقي رعاية وظروف صحية وتغذية جيدة والمجموعة الثانية تتكون من ٥٣ أنثي غنم تربي في قطعان أهلية صغيرة وتعاني من رعاية وظروف صحية وتغذية غير جيدة) والمجموعة الثالثة عبارة عن خمسين أنثي ماعز تربي في قطعان أهلية صغيرة وتعاني أيضا من رعاية وظروف صحية وتغذية غير جيدة. تم تجميع ٣١٣ عينة لبن بواقع ١١٨ عينة من المجموعة الأولي، ١٠١ عينة من المجموعة الثانية و٩٤ عينة من المجمّوعة الثالثة. اعتبرت الغدة اللبنية مصابة بالتهاب الضرع الخفي علي أساس ايجابية العينة لكل من اختبار الكاليفورنيا والفحص البكتريولوجي، وقد كانت نسبة انتشار التهاب الضرع الخفي ٥,٠٨% في المجموعة الأولي، ١٦,٨٣% في المجموعة الثانية، ٣٧,٢٣% في المجموعة الثالثة. أوضحت الدراسة أنه هناك فروق معنوية عالية جدا" (P >0.001) في معدل الإصابة بالتهاب الضرع الخفي في المجموعتين الثانية والثالثة عنها في المجموعة الأولي. كما أوضحت الدراسة أن الميكروب العنقودي السالب لأنزيم االكواجيليز من أكثر الميكروبات المعزولة من الحالات المصابة بالتهاب الضرع الخفي (٤٨,١٥% و٤١,٨٦% في إناث الغنم والماعز، علي الترتيب). تم أيضا عزل الميكروب المكور العنقودي الذهبي بنسبة ٢٢,٢٢% و٩,٣٠% والميكروب القولوني بنسبة ١٨,٥٢% و٣٠,٢٣% والميكروب المكور السبحي بنسبة ٧,٤١% و١٣,٩٥%، من إناث الغنم والماعز علي الترتيب. وبإجراء اختبار الحساسية للمعزولات معمليا" تبين أنها كانت حساسة لكل من الأفلوكساسين وسيبروفلوكساسين وجنتاميسين وأوكسي تتراسيكلين بنسبة ١٠٠%، ٩٨,٢٨%، ٨٤,٤٨%، ٨٤,٤٨% علي التوالي. كما أظهرت أغلب العترات مقاومة لكل من البنسلين وسيفوتكسيوم والأمبيسلين. ويمكن الخلاصة بأن: ١- انتشار التهاب الضرع الخفي بين إناث الماعز أكثر من انتشاره بين إناث الأغنام. ٢- الميكروب المكور العنقودي وبخاصة المكور العنقودي السالب لأنزيم االكواجيليز من أكثر المسببات البكترية لالتهاب الضرع الخفي في المجترات الصغيرة. ٣- البكتريا المعزولة لها حساسية عالية لعدد من المضادات البكترية. ٤- انتشار التهاب الضرع الخفي في المجترات الصغيرة يتأثر بالظروف الصحية والرعاية والتغذية التي تقدم للحيوان.