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PREVALENCE AND ETIOLOGY OF SUBCLINICAL MASTITIS IN DAIRY EWES AT FAYOUM GOVERNORATE, EGYPT

(With 6 Tables)

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معدل إنتشار ومسببات إلتهاب الضرع الكامن فى الأغنام فى محافظة الفيوم- مصر

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خلال هذه الدراسة تم فحص عدد ١٩٦ عينة لبن من ١٦٣ غنمة حلوب بمحافظة الفيوم وذلك لدراسة بعض الجوانب المتعلقة بإلتهاب الضرع الكامن فى الأغنام. بإستخدام إختبار شالم وكذلك الفحص البكتريولوجى تم الكشف عن إلتهاب الضرع الكامن فى عدد ٤٨ غنمة بنسبة ٢٩,٤٥% وعدد ٦٢ نصف ضرع بنسبة ٣١,٦٣ %. كان معدل الإصابة فى الأغنام التى فى مواسم الحلابة المتعددة (٣٢,٠٣%) أكثر مقارنة بالأغنام التى فى موسمها الحلابى الأول (٢٠%) لكن هذه الزيادة ليست معنوية إحصائيا. وقد أظهر أيضا إختبار شالم معدل إصابة مرتفعه (٥٥,٦١%) مقارنة بالعزل البكتريولوجى (٣٩,٨٠%). كان الميكروب العنقودى السالب لإختبار الكوأجيليز من أكثر الميكروبات المعزولة حيث تم عزله بنسبة ٥٠% من العترات المعزولة من عينات موجبة لإختبار شالم ونسبة ٤٨,٧٢% من إجمالى العترات المعزولة ثم جاء بعد ذلك الميكروب العنقودى الموجب لإختبار الكوأجيليز. تم أيضا عزل الميكروب السبحى وميكروب الإيشيريشيا كولاى بنسب مختلفة. بالإضافة إلى ذلك تم الكشف عن خمائر لم يتم تصنيفها بنسبة ٤,٨٤% من العترات المعزولة من عينات موجبة لإختبار شالم ونسبة ٥,١٣% من إجمالى العترات المعزولة. وبقياس مستوى أنزيم اللاكتات دى هيدروجينيز وأنزيم الالكالين فوسفاتيز تبين إرتفاعهم بصورة ملحوظة فى لبن الأغنام المصابة بإلتهاب الضرع الكامن مقارنة بالأغنام السليمة. على العكس من ذلك لم يختلف مستوى هذان الإنزيمان فى مصل الدم للأغنام المريضة والسليمة.

SUMMARY

In Fayoum Governorate, a total of 196 milk samples were collected aseptically from apparently healthy functioning glands of 163 dairy ewes to study subclinical mastitis (SCM) in dairy ewes. According to the

definition of SCM the only sample that showed California Mastitis Test (CMT) and bacteriology positive results was considered to have subclinical mastitis, the prevalence of SCM was 29.45% in regard to ewes and 31.63% in regard to glands. Subclinical mastitis was higher in multiparous ewes (32.03%) than primiparous ones (20.0%), but this increase is not statistically significant. CMT was useful as a screening test in ovine species to identify infected animals, keeping in mind that the test showed higher prevalence rate of subclinical mastitis than bacteriological culture (55.61% compared with 39.80%). Staphylococci were the most common bacteria detected (73.08% isolates), where coagulase-negative staphylococci (CNS) were detected in 50% of CMT positive samples isolates and in 48.72% of the total bacterial isolates and *Staphylococcus aureus* (*S. aureus*) was isolated in a percentage of 30.65% only from CMT positive samples followed by *Streptococcus agalactiae (S. agalactiae)* in 9.68% of CMT positive samples isolate and lastly *Escherichia coli (E. coli)* in 4.84% of CMT positive samples isolate and in 14.10% of the total bacterial isolates. Yeast was also detected in 4.84% of the CMT positive samples isolate and in 5.13% of the total isolates. Enzymatic activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) proved to be good indicator for intramammary infection in ewes, where, enzymatic activities of LDH and ALP were significantly higher ($p < 0.05$) in milk from subclinically mastitic ewes compared to milk from healthy ones. On the other hand, there were no significant alterations recorded in the levels of serum LDH and ALP of subclinically mastitic ewes compared to healthy ones.

Key words: *Mastitis, subclinical mastitis, dairy ewes, milk*

INTRODUCTION

Mastitis is one of the most serious health and economic problems in all dairy sheep flocks all over the major sheep breeding countries (Fthenakis and Jones, 1990 and Kirk and Glenn, 1996). Although ovine clinical mastitis is typically gangrenous and causes death, the more important economically is subclinical mastitis due to its high prevalence rate (Marco, 1994) and association with decrease in milk production in addition to growth retardation and high mortality rate among lambs in suckling ewes (Gross *et al.*, 1978; Waston and Buswell, 1984; McCarthy *et al.*, 1988; Fthenakis and Jones, 1990; Mavrogenis *et al.*, 1995; Dario *et al.*, 1996; Peris *et al.*, 1996 and Saratsis *et al.*, 1999).

Intramammary infections (IMI) in dairy small ruminants are mainly of bacterial origin by either contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) or environmental pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus uberis* and coagulase-negative staphylococci) (Radostits *et al.*, 2000 and Bergonier *et al.*, 2003). Staphylococci are the main etiological agents of small ruminants intramammary infections (IMI), where, *Staphylococcus aureus* is the most common pathogen isolated in clinical cases and coagulase-negative staphylococci (CNS) are the most prevalent in subclinical cases (Fthenakis, 1994; Lafi *et al.*, 1998 and Bergonier *et al.*, 2003).

Diagnosis of subclinical mastitis is commonly based on cytological examination as well as biochemical changes in milk but confirmation must be based on bacteriological examination (Keisler *et al.*, 1992 and Radostits *et al.*, 2000)

The aim of the present work was directed to determine the prevalence of subclinical mastitis in dairy ewes and the microbial agents associated with such infections. In addition, to study the changes occurring in the levels of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in the milk and blood as a result of subclinical mastitis in this species.

MATERIALS and METHODS

Animals:

In this study, 163 native dairy ewes (Balady breed) from 17 flocks belonging to Fayoum Governorate, Egypt were used to study some aspects concerning subclinical mastitis in this species. Ewes selected for this investigation were apparently healthy, free from any signs of clinical mastitis and other palpable udder lesions. The system of milking in these flocks was hand milking.

Sampling Procedure:

A total of 196 milk samples were collected aseptically from apparently healthy functioning glands of 163 dairy ewes according to the method described by Al-Majali and Jawabreh (2003) and Batavani *et al.* (2003) as follows: ewes were restrained in a sitting position and the teat end of each udder half was scrubbed thoroughly using cotton wool soaked in 70% ethyl alcohol. The first three streams were discarded, the teat orifice was disinfected again as described and 10 ml milk sample was taken from each gland in a sterile tube and examined for any macroscopic abnormalities by visual inspection and strip cup method. In

addition, jugular blood samples (10 ml) were taken by vein puncture form each animal into dry centrifuge tubes without anticoagulant and allowed to clot. All samples were kept at 4°C during transportation and delivered to the laboratory for examination within 2 to 4 hours after collection. Serum was separated after centrifugation and stored at -20°C until thawed for analysis. All milk and blood samples were collected in mid-lactation (2^{nd} week after lambing until 10^{th} week postpartum)

California Mastitis Test (CMT):

CMT was carried out for all milk samples, using the method described by Schalm *et al.* (1971). According to the visible reactions, the results were classified in five degrees of reaction scores: 0 = negative, trace, 1= weak positive, 2 = distinct positive and 3 = strong positive.

Bacteriological Examinations:

All milk samples (10 µl from each) were inoculated onto the surface of 5% sheep blood and MacConkey agar plates. All plates were incubated aerobically at 37°C and examined for growth at 24 hours. Hemolysis and colonial morphology were recorded after 24 - 48 hours. Gram positive cocci with catalase positive and oxidase negative reaction were subjected to coagulase test by tube method (Langlois *et al.*, 1990). Other bacterial isolates were examined for their staining affinity, morphological, cultural and their biochemical characters according to the standard methods described by Cruickshank *et al.* (1975).

CAMP test:

Staphylococcus aureus was streaked across the center of sheep blood agar. A streak of the suspected *Streptococcus* is made at right angle too and taken within 1 – 1.5 mm of the staphylococcal streak. Plate incubated at 37° C for 18-24 hours. An arrow head formed at junction between streaks means positive CAMP test (Cruickshank *et al.*, 1975).

Sodium hippurate hydrolysis test:

Used for confirmation of *Streptococcus agalactiae*. 1% aqueous solution of sodium hippurate that inoculated with the suspected microorganism was incubated at 37°C for two hours, then 0.2 ml of a ninhydrin solution was added. The positive reaction is given by a deep purple color developed after 10 minutes.

Enzyme Analysis:

All milk samples were centrifuged at 3000 r. p. m. for 10 minutes and defattened milk was prepared. LDH and ALP activities were measured in blood serum and defattened milk according to the method described by Bergmeyer (1974).

Statistical analysis:

Chi-square and student '*t* test were carried to the obtained data according to Snedecor and Cochran (1980). Probabilities less than 0.05 were considered significant.

RESULTS

In the present study, out of the examined 196 milk samples which collected aseptically from apparently healthy functioning glands of 163 dairy ewes, Positive CMT was recorded in 109 (55.61%) of the examined glands and in 88 (53.98%) of the examined ewes. Bacteria and yeast were isolated from 78 (39.79%) of the examined udder halves and 59 (36.20%) of the examined ewes. So, using the definition of SCM as the presence of both bacteriologically positive and CMT positive results, 62 (31.63%) of the examined glands and 48 (29.45%) of the examined ewes were affected (Table 1 and 2).

Table 1: Results of CMT and bacteriological culture on examined milk samples (N=196).

Bacteriology / CMT	CMT + ve		CMT – ve		Total	
	No.	%	No.	%	No.	%
CMT + ve	62	56.88	47	43.12	109	55.61
CMT – ve	16	18.39	71	81.61	87	44.39
Total	78	39.80	118	60.20	196	100

Samples that gave score 1 were considered positive for CMT

Table 2: Results of CMT and bacteriological culture on examined ewes (N=163).

Bacteriology / CMT	CMT + ve		CMT – ve		Total	
	No.	%	No.	%	No.	%
CMT + ve	48	54.54	40	45.45	88	53.99
CMT – ve	11	14.67	64	85.33	75	46.01
Total	59	36.20	104	63.80	163	100

Subclinical mastitis was higher in multiparous ewes (32.03%) than primiparous ones (20.0%), but this increase is not statistically significant as shown in Table 3.

Table 3: Prevalence of subclinical mastitis in relation to parity status.

Animal status	No. of examined ewes	No. of infected ewes	%
Primiparous ewes	35	7	20.0
Multiparous ewes	128	41	32.03
Total	163	48	29.45

Frequent distribution of the microbial isolates detected in bacteriology positive milk samples in dairy ewes as shown in Table 4 were: CNS in 48.72% of the total isolates 50% of them from CMT positive samples, *Staphylococcus aureus (S. aureus)* and *Streptococcus agalactiae* (*S. agalactiae*) were detected only from CMT positive samples in 30.65% and 7.69% respectively, *Escherichia coli (E. coli)* in 14.10% of the total isolates 4.84% of them from CMT positive samples and unidentified yeast species in 5.13% of the total isolates, 4.84% of them from CMT positive samples..

Table 4: Frequency of bacterial isolates in milk obtained from udder halves which demonstrated positive and negative reactions to the CMT.

Microorganisms	CMT + ve samples (N=62)		CMT – ve samples (N=16)		Total isolates (N=78)	
	No.	%	No.	%	No.	%
CNS	31	50.0	7	43.75	38	48.72
S. aureus	19	30.64	0	0.0	19	24.36
S. agalactiae	6	9.68	0	0.0	6	7.69
E. coli	3	4.84	8	50.0	11	14.10
Unidentified yeast	3	4.84	1	6.25	4	5.13

Staphylococci (CNS and *S. aureus*) were the most common bacteria detected (73.08%).

Enzymatic activities of LDH and ALP in milk and serum of healthy and subclinically mastitic ewes are shown in Tables 5 and 6.

Table 5: Mean ± SE levels of LDH and ALP in examined milk samples (IU/l).

	Normal milk (N=20)	Subclinical mastitic milk (N=30)
LDH	610.11 ± 17.38	886 ± 41*
ALP	100.09 ± 15.17	143 ± 11*

* = Significant at $P < 0.05$ *vs.* controls.

Table 6: Mean ± SE levels of LDH and ALP in examined serum samples (IU/ I).

	Serum from healthy ewes	Serum from infected ewes
LDH	730. 11 ± 100.04	766 ± 89.1
ALP	149.15 ± 118.70	136 ± 17.1

There was no significant differences in the levels of LDH and ALP in blood serum.

DISCUSSION

Mammary glands without clinical abnormalities and giving apparently normal milk, but with bacteriological counts higher than 200 CFU/ml of the same type of colonies and with positive CMT were considered to have subclinical mastitis (Stefanakis *et al.*, 1995).

In this study, the prevalence of subclinical mastitis in native dairy ewes in regards to ewes was 29.45%. Similar results were reported previously by Kirk *et al.* (1996) who recorded a prevalence rate of 29%. Higher prevalence was reported by Winkler and Gootwine (1989) who recorded prevalence rate of 55%. Lower prevalence was reported by Ahmed *et al.* (1992) and Al-Majali and Jawabreh (2003) who reported prevalence rates of 25% and 18.3% respectively.

Concerning the prevalence of subclinical mastitis in regards to glands, it was 31.63%. Lower prevalence was reported previously by Kirk *et al.* (1996) and Al-Majali and Jawabreh (2003) who recorded prevalence rates of 13.1% and 10.4% respectively. In contrast, higher prevalence rates were reported by Ariznabarreta *et al.* (2002) and Batavani *et al.* (2003) who reported prevalence rates ranged from 39 to 41%. These variations in the disease prevalence may be attributed to the differences in the husbandry, management, nutrition, size of flock, breed, parity of the lamb, lactation period, season and the used diagnostic criteria (McCarthy *et al.*, 1988; Tietze *et al.*, 1993; Fthenakis, 1994; Stefanakis *et al.*, 1995; Dario *et al.*, 1996 and Lafi *et al.*, 1998).

In this study, Subclinical mastitis was higher in multiparous ewes (32.03%) than primiparous ones (20.0%), but this increase is not statistically significant. Similar observations were recorded previously by Watkins *et al.* (1991) and Lafi *et al.* (1998). This may be attributed to the cumulative stress on the mammary tissues or to increased prevalence of infection and permanent glandular damage from previous infections (Lafi *et al.*, 1998).

CMT test showed higher prevalence rate of subclinical mastitis than bacteriological culture (55.61% compared with 39.80%). This may

be explained on the fact that CMT has been standardized for cow's milk so, it is most accurate in this species (Schalm *et al.*, 1971).

CMT was useful as a screening test in ovine species to identify animals for culture, keeping in mind that certain selected animals will be culture negative (false positive results). These false positive results may be attributed to the presence of non infectious factors that can affect milk somatic cell count as diurnal variation of SCC (up to 40% increasing in evening milking than that obtained in morning milking) and the stage of lactation (Fthenakis, 1996). In addition, ewes tended to have a higher cell counts, nuclear fragments, cytoplasmic particles and fat content in normal milk (Green, 1984; Maisi *et al,* 1987; Fthenakis and Jones, 1990 and Donovan *et al.*, 1992).

Staphylococci were the most common bacteria detected in this study representing a total percentage of 73.08% of positive cultures, with CNS representing 48.72% from these isolates. Similar findings were reported in previous studies by De la Cruz *et al.* (1994); Gonzalez–Rodriguez *et al.* (1995); Las Heras *et al.* (1999); Menzies and Ramanoon, (2001); Vieira–da-Motto *et al.* (2001); Ariznabarreta *et al.* (2002); McDougall *et al.* (2002); Bergonier and Berthelot (2003) and Bergonier *et al.* (2003) who recorded that CNS were the most prevalent pathogens of the mammary gland in sheep. The highest infection rate by CNS may be explained by the fact that CNS is encountered in the environment as an environmental pathogen. So; it is able to colonize the skin of animal, introduced from the skin to the gland by the process of suckling or during milking via teat canal causing minor mastitis in ewes. The CNS have been reported to produce virulence factors hence clinical infections are being increasingly associated with the organisms (Nobel, 1992). The virulence factors may be responsible for the cases of clinical and subclinical mastitis due to CNS (Jarp, 1991; Devriese *et al.*, 1994 and Lafi *et al.*, 1994). *Staphylococcus epidermidis* has been described as a potential pathogen for the mammary gland; either increasing SCC or causing persistent infections. In addition, *S. epidermidis* is commonly considered to be a mastitis pathogen of low virulence (Fthenakis and Jones, 1990 and Burriel, 1997).

Teat dipping which is an integral part of mastitis control programs in dairy flocks was not practiced in any one of the flocks under study. So, the increasing in the environmental and opportunistic pathogens is mainly related to poor conditions of environmental hygiene and/or decreased defenses of the mammary gland (Moroni and Cuccuru, 2001 and Albenzio *et al.*, 2002). Similar results were observed

previously by Harmon and Langlois (1995) who recorded that the prevalence of *Staphylococcus* spp. appeared to be influenced by teat dipping. Furthermore, Hogan *et al.* (1987) found that *S. epidermidis* was the predominant staphylococci for lactating dairy cows when teat dipping was not practiced.

Staphylococcus aureus was the second most common pathogen isolated in a percentage of 30.64% from CMT positive samples isolates and in percentage of 24.36% from the total bacterial isolates. The high persistence of mastitis due to *S. aureus* is related to its capacity to produce exo-polysaccharides (''slime''), which form a protective barrier that restricts the efficiency of both the immune responses and chemotherapy (Baselga *et al.*, 1994).

The percentage of *Streptococcus agalactiae* in this study was 9.68% in CMT positive samples isolate and in a percentage of 7.69% from the total bacterial isolates, in accordance with the results reported previously by Gnozalez–Rodriguez *et al.* (1995) who recorded a percentage ranged from 8.2% to 18%.

Escherichia coli was detected in a percentage of 4.84% of CMT positive samples isolates and in a percentage of 14.10% from the total bacterial isolates. Similar results were recorded previously by Maisi *et al.* (1987) and Lafi *et al.* (1994).

In addition to the above mentioned bacteria, yeast was detected in 4.84% of the CMT positive samples isolate and in 5.13% from the total isolates. These results are in agreement with the results previously reported by Gonzalez-Rodriguez *et al.* (1995) who isolated yeast from ewes having subclinical mastitis.

Concerning the use of enzymatic changes that occurred in milk as an assisting tool in diagnosing of subclinical mastitis, LDH and ALP have been used in cattle to diagnose udder infections (Kovac and Beseda, 1975; Michel, 1979; Kitchen, 1981; Deianov, 1983 and Pednekar *et al.*, 1992). In this study measuring of the LDH and ALP revealed that the mean activity of LDH and ALP were significantly higher ($p < 0.05$) in milk from subclinically mastitic milk compared to those of normal milk. Similar result was reported previously by Batavani *et al.* (2003). Higher LDH activity in milk of subclinical mastitic ewes than that in controls has previously reported by Nizamlioglu *et al.* (1989) and Nizamlioglu and Erganis (1991). Intramammary infection increases the permeability of microcirculatory vessels by secretion of various chemical mediators such as histamine, prostaglandin, kinins and free oxygen radicals from inflammatory cells (Honkanen-Buzalski and

Sandham, 1981). So, changes in LDH and ALP activities might be suitable for early diagnosis of subclinical mastitis in ewes. The higher level of LDH in mastitic milk than blood serum LDH activity shows that blood serum was not the sole source of this enzyme in mastitic milk and it was probably also liberated from udder parenchymal cells and from disintegrated leucocytes (Michel, 1979; kitchen, 1981; Deianov, 1983; and Kato *et al.*, 1989).

Finally it can be concluded that, subclinical mastitis was more prevalent in dairy ewes, staphylococci especially CNS were the most prevalent pathogen and CMT was useful as a screening test in ovine species but false positive results may occurred and so bacteriological culture must be done for accurate diagnosis. Moreover, LDH and ALP activities in milk might be an assisting tools for early diagnosis of intramammary infection in ewes.

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