

## Study on Frequency, Aetiology and Some Enzymatic Activities of Subclinical Ovine Mastitis in Urmia

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### Summary

A total of 209 milk samples were collected from the udder halves of 178 native dairy ewes at 2 weeks after lambing until the 10th week postpartum. Those, which were classified by bacterial culture and California Mastitis Test (CMT) as positive, were deemed to have glands with subclinical mastitis (SCM). The periodic prevalence rate of SCM was 39%. The most common bacterial isolates from SCM cases were coagulase negative staphylococci (41%), *Bacillus cereus* (33%), *Staphylococcus aureus* (22%) and *Streptococcus* spp. (4%). The mean activity of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were higher in milk from SCM udders than in milk from healthy udders ( $p < 0.01$ ). There were no significant differences in blood serum LDH, ALP and aspartate aminotransferase (AST) of healthy and subclinical mastitic ewes. The increment in LDH and ALP in milk of udders shows the presence of tissue damage provoked by SCM. Thus, these parameters might be suitable for use in the early diagnosis of subclinical mastitis in ewes.

**Keywords:** subclinical mastitis; sheep; aetiology; prevalence, enzyme

### Introduction

Mastitis is inflammation of the mammary gland. Inflammation is most commonly caused by infection with a pathogen, but it may also be caused by injury and less commonly by allergy and neoplasm (Menzies & Ramanoon 2001). Traditionally,

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*Staphylococcus aureus* and *Pasteurella haemolytica* have been regarded as the principal cause of mastitis in ewes. *Escherichia coli*, *Streptococcus* spp. and coagulase negative staphylococci (CNS) cause most of the remaining. Other bacteria incriminated sporadically include *Arcanobacter pyogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Closteridium preferingens*, *Listeria monocytogens*, *Actinobacillus* spp., and *Mycoplasma agalactiae*. *Clamidia psittaci* mastitis has been produced experimentally in sheep, but there is no evidence that it causes mastitis in field circumstances. One non-bacterial causes of mastitis is the maedivisna virus, which can cause indurative lymphocytic mastitis in ewes (Jonse & Watkins 2002). Sheep have always been of vital economic importance as producers of meat, wool and milk in many countries. Clinical mastitis in sheep has generally been considered as a main source of financial loss, but the economic loss caused by subclinical mastitis is also important. Clinical ovine mastitis is typically gangrenous and causes death, subclinical mastitis results in decreased milk yield in sheep and in consequent growth retardation and higher mortality rate among lambs in suckling ewes (Watson & Buswell 1984). The aims of our investigation were (a) to determine the occurrence rate and aetiology of subclinical mastitis in sheep in Urmia, (b) to study the changes occurring in the level of lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase in the milk as a result of subclinical mastitis in the udder.

### **Materials and Methods**

**Animals.** The study was carried out on 178 primiparous and pluriparous native dairy ewes from 12 flocks in Urmia province in west Azerbaijan of Iran. All flocks grazed during spring and summer with little feed supplementation. Ewes were usually housed during fall and winter and fed wheat straw, alfalfa, barley grain and wheat bran. These animals lambed between February to March 1999 and their lambs were kept with their dams for 120-150 days the time of drying-off. Ewes selected for this investigation were apparently health, free of clinical mastitis and any other palpable udder lesions.

**Sampling procedure.** Ewes were restrained in a sitting position and the teat end of each half udder was scrubbed thoroughly using cotton wool soaked in 70% ethyl alcohol. The three first streams were discarded, the teat orifice was disinfected again as described and 5ml milk samples were taken in a sterile tube held horizontally. In addition jugular blood samples (5ml) were taken by venipuncture from each animals. All samples were kept cold during transportation and delivered to the laboratory for examination within 2h after collection. All milk and blood samples of ewes were tested in mid-lactation (at 2 weeks after lambing until 10th week postpartum) and none of the ewes were sampled twice in the study.

**CMT.** California Mastitis Test (CMT) was carried out in all milk samples, using the method by Schalm *et al* (1971). According to the visible reactions the results were classified in four scores: 0=negative or trace, 1=weak positive, 2=distinct positive and 3=strong positive.

**Bacteriological examinations.** All milk samples requiring bacterial culture were mixed well and a standard loopful (0.01ml) from milk sample was inoculated on the surface of blood agar (Bacto-Agar, Difco) containing 5% of washed sheep red blood cells, and MacConkey agar plates. All plates were incubated aerobically at 37°C and examined for growth at 24h. If there was no growth, the plates were reincubated and the final assessment was made at 48h. The presence of 6 or more colonies of the same type was considered to be significant and the sample was recorded as positive. The bacteria were identified by using Sears *et al* (1993) and Quinn *et al* (1994) descriptions.

**Enzyme analysis.** Blood serum and defatted milk (centrifuged milk at 3000g for 10 min) used for enzyme estimations. Determination of aspartate aminotransferase, and lactate dehydrogenase and alkaline phosphatase were done by the method of Reitman and Frankel (1957), and Begmeyer (1974) respectively.

**Statistical analysis.** Statistically significance associations between CMT and culture were determined by chi-squared distribution (MacNemar's test) and value with

$P < 0.001$  were considered as significant. Specificity and sensitivity of CMT test were calculated by formulae (Petrie & Watson 1999). Results of enzymes were given as mean  $\pm$  SEM and student's  $t$  test with  $P < 0.01$  was used to evaluate differences between subclinical mastitic and healthy ewes.

### Results

During the study period, 209 milk samples were collected from functioning glands of 178 ewes. Positive CMT were recorded from 148 (71%) glands. Bacteria were isolated from 87(49%) ewes and 107 (51%) udder halves. Using the definition of SCM as the presence of both a bacteriologically positive and CMT positive results, 81(39%) glands were affected. The specificity and sensitivity of CMT test in detecting subclinical mastitis were 34.31% and 75.70 %, respectively (Table 1). Distributions of microbial isolates responsible for subclinical udder infection were: coagulase negative staphylococci (41% of isolates), *Bacillus cereus* (33%), *Staphylococcus aureus* (22%) and *Streptococcus* spp. (4%).

Table 1. Two-way contingency table to show the relationship between bacteriological and California mastitis test results for 209 milk samples

	Culture(+)	Culture(-)	Total
CMT(+)	81	67	148
CMT(-)	26	35	61
Total	107	102	209

$$\chi^2_1 = 17.20 > \chi^2_2, 0.999 = 10.83$$

Table 2 shows the activities of LDH, ALP and AST in normal and subclinical mastitic milk and serum samples. The mean LDH and ALP activities were significantly higher ( $P < 0.01$ ) in milk from inflamed (SCM) udders than in normal milk, but there were no significant difference in AST values. There were no significant differences in blood enzyme values.

### Discussion

Literature pertaining to ovine subclinical mastitis in different breeds of sheep in Iran is very limited. However, several studies in the world have been conducted for the assessment of the occurrence of ovine udder infection in other breeds of sheep. According to the results of the present study, the occurrence rate of subclinical mastitis in native sheep in Urmia seems to be higher than that reported by Watkins *et al* (1991), but it is agreement with ranges published by Keisler *et al* (1992) and Leitner *et al* (2001). A direct comparison between these results is difficult, because of differences in the management, nutrition, size of flock, breed, parity of the dam, lactation period, season, case definition and the diagnostic criteria used. Effect of above-mentioned factors has been reported on the prevalence of subclinical mastitis (McCarthy 1988, Fthenakis 1994, Stefanakis *et al* 1995, Burriel 1997, Lafi *et al* 1998).

Table 2. Changes in the level of LDH, ALP, and AST (IU/L) in milk and serum as a result of subclinical mastitis in ewes

Enzyme	Milk		Serum	
	Normal	Subclinical mastitic	Normal	Subclinical mastitic
LDH	627.71± 28.43 (50)	981.21± 45.98 (57)	719.04±105.30 (17)	900.93±109.28 (15)
ALP	102.09± 18.10 (50)	172.66± 19.44 (57)	142.12±16.96 (17)	142.32±29.07 (15)
AST	59.53± 4.08 (15)	54.90± 2.12 (40)	32.83±1.79 (17)	34.27±1.81 (15)

Parenthesis shows the number of samples

In this study CMT test showed higher prevalence rate of subclinical mastitis than bacteriological culture (71% compared with 51%). The CMT has been standardized for cow's milk and is most accurate in this species. Ewes tended to have a higher cell counts, nuclear fragments, cytoplasmic particles and fat content in normal milk (Donovan *et al* 1992). CMT only reacts with liberated nuclear DNA. CMT is a

subjective test. It may be useful as a screening test in ovine species to identify animals for culture, keeping in mind that a high percentage of animals selected will be cultured negative. Other researchers also reported that the field use of the CMT for detecting intramammary infection (IMI) in sheep was less reliable and demonstrated that the capability of CMT to predict ovine IMI depends on the prevalence and the agents of IMI in the flock (Hueston *et al* 1986b, Keisler 1992).

The most frequent isolate from subclinical cases was CNS. These organisms have been considered to be the major cause of non-clinical intramammary infections in a number of previous investigations (Hueston *et al* 1986a, Burriel, 1997 and 1998, Lafi *et al* 1998, Leitner *et al* 2001). Generally, these organisms had been considered as a nonpathogenic or of low pathogenicity for the mammary gland of domestic ruminants. However, some species have been shown to raise inflammatory indicators as effectively as the coagulase positive staphylococci. Recently researchers proposed CNS as aetiological agents of subclinical mastitis in goats (Poutrel 1984) and cattle (Smith & Hagstad 1986, Waage *et al* 1999). CNS organisms are present in the air and dusts of occupied buildings are easily transferred between hosts. CNS encountered in the environment of ewes is able to colonize the skin of animal and can be introduced from the skin to the gland by the process of suckling or during milking and if pathogenic, they may cause SCM. Other isolated bacteria were followed by *Bacillus cereus*, *Staphylococcus aureus* and *Streptococci* spp. In addition to above-mentioned bacteria, other workers had reported *Arcanobacter pyogenes* (formerly *Actinomyces pyogenes*) (Stefenakis *et al* 1995), *E.coli* (Lafi *et al* 1998) and *Pasteurella hemolytica* (Watkins *et al* 1991, Al-Ani *et al* 1997) as aetiological agents of subclinical mastitis in sheep.

Enzyme changes have been used to diagnose udder infections in cows (Kitchen 1981, Deianov 1983, Pednekar *et al* 1992), but little information was available in relation to changes in enzyme level in blood or biological fluids for many commonly encountered sheep diseases of which ovine mastitis was noted as being an important. Our findings showed that the mean activity of LDH and ALP were higher in milk

from subclinical mastitic udders than in normal milk. Change in LDH activity of subclinical mastitic ewes' milk has previously reported (Nizamlioglu *et al* 1989, Nizamlioglu & Erganis 1991). Inflammation of the mammary glands increases the permeability of microcirculatory vessels by secretion of various chemical mediators such as histamine, prostaglandin, kinins and free oxygen radicals from inflammatory cells. 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 leukocytes (Kitchen 1981, Deianov 1983). Unfortunately, no published report was available about ALP and AST levels of ovine milk to compare the present findings. Bogin and Ziv (1973) found approximately a 6-fold increase in the level of ALP after infusion of *E. coli* endotoxin into the bovine udder. The exact origin of the increased ALP activity in mastitic milk has not yet been determined. The pattern of distribution of AST in the milk of normal and inflamed udder showed no significant differences and AST of milk was higher than the level found in blood serum. They proposed that AST in bovine milk was essentially a blood serum derived enzyme. However, a considerable amount of AST in homogenates from healthy mammary gland tissue suggests that a major source of AST in normal and mastitic milk is the mammary gland secretory cells.

In conclusion, the present study indicated that 1) CMT has limited value for diagnosing SCM in ewes. Therefore, direct microbiological culture of milk is required for accurate diagnosis and epidemiological surveillance. 2) Coagulase negative staphylococci are predominant cause of subclinical mastitis. 3) The higher LDH and ALP activities in milk from SCM udders appeared to be an indicator of intramammary infection in ewes.

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