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# Prevalence and Etiology of Subclinical Mastitis in Ewes of the Tabriz Region, Iran

[1]Rahim Beheshti, [1]Jalal Shaieghi, [1]Behrad Eshratkhah, [2]Jamshid Ghiasi Ghalehkandi and [2]Naser Maheri-Sis

[1]Department of Clinical Sciences,Veterinary Faculty, Islamic Azad University,
Shabestar Branch, 53815-159 Shabestar, Iran
[2]Department of Animal Science, Islamic Azad University,
Shabestar Branch, 53815-159 Shabestar, Iran

**Abstract:** To investigate the periodic prevalence, etiology and some epidemiological features of subclinical mastitis in ewes from the Tabriz region, milk samples from 260 lactating ewes were aseptically collected for bacterial and California mastitis test (CMT). An association was observed between the occurrence of subclinical mastitis and the age of ewe. The periodic prevalence rate of SCM was 9.23%. *Staphylococci* were the most prevalent bacteria, representing 88.4% of the isolates. Coagulase-negative staphylococci (CNS) (69.2%), was the most prevalent species followed by *staphylococcus aureus* (19.2%). *Escherichia coli* and *Corynebacterium bovis* was the second bacterial group in importance according with the distribution among flocks representing 8% and 8% of the isolates. In conclusion, subclinical mastitis seems to be, as deduced from the high prevalence observed in this study, an important health problem for milking sheeps in the Tabriz region.

**Key words:** Subclinical mastitis · Sheep · Etiology · Prevalence

## INTRODUCTION

Mastitis, which is inflammation of the mammary gland, is among the most important diseases in dairy herds [1]. Although clinical cases of mastitis are a source of loss, more important economically is subclinical mastitis due its higher prevalence and associated decrease in milk production [2]. Although subclinical mastitis occurs worldwide, its economical importance is especially significant in the Mediterranean countries, because these are the highest sheep milk producers within the EU [2]. Few researchers have studied the incidence and the consequences of the subclinical form of the disease.

However, Madrid is important region in sheep milk, data about prevalence of subclinical mastitis in dairy sheep flocks in this region are lacking or limited. Subclinical mastitis reduces milk production and quality and may contribute to lamb mortality. The importance of subclinical mastitis as a limiting factor in milk production in cows is well documented [3]. In addition to reduction of milk yield, lower lamb performance and higher predisposition to clinical mastitis [4], subclinical mastitis has also adverse effects on the hygienic quality and physicochemical properties of milk [5]. The limits of somatic cell counts of sheep milk, have not yet been definitely established, although it has been suggested that a threshold level for subclinical mastitis in sheep should be close to 1,500 000 cell $mL^{-1}$, much higher than that set for cows (<500,000 cell $mL^{-1}$) [6]. In Iran, the prevalence of subclinical mastitis has been studied in Urmia regeon, showing, in general, relatively high rates [7]. The association between subclinical and clinical mastitis in sheep is not fully understood, although one report suggests that an association does not exist [8]. The objective of this investigation was to determine the prevalence and etiology of subclinical mastitis and it's relationship with CMT in sheep.

## MATERIALS AND METHODS

**Flocks and Survey Design:** Milk samples from 260 ewes from 12 flocks, selected by stratified random sampling, located in flocks in Tabriz province in west Azerbaijan of Iran. Within each flock ewes that had lambed recently were randomly selected and sampled. Identity, age and parity were recorded. Abnormalities on the udder were recorded.

**Corresponding Author:** Rahim Beheshti, Department of Clinical Sciences, Veterinary Faculty, Islamic Azad University, Shabestar Branch, 53815-159, Shabestar, Iran

**Milk Sampling and Bacteriological Procedures:** Samples were collected between March and May of 2007. Udders and mammary secretions were examined for macroscopic signs of abnormality. Milk samples (5 ml) were taken aseptically, prior to the morning milking, from each mammary gland after cleaning the teat end with cotton soaked in 70% ethyl alcohol and previous discard of the first three streams of milk. Samples were kept at 4°C during transportation to the laboratory for bacteriological analysis which was carried out 2 h after collection. All milk samples requiring bacterial culture were mixed well and a standard loopful (0.01 ml) from each milk sample was inoculated on the surface of blood agar 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 24 h. If there was no growth, the plates were reincubated and the final assessment was made at 48 h. The presence of six or more bacterial colonies of the same type on the medium was considered to be significant and the samples was recorded as positive. Bacteria were identified by using colony morphology, hemolytic pattern on blood agar media and further microscopic examination (Gram staining), standard biochemical methods (catalase, haemolysis, coagulase test with rabbit plasma) described by Quinn *et al*. [9].

**Somatic Cell Count:** The California Mastitis Test (CMT) was applied to all samples collected using the method of Schalm *et al*. [10]. According to the reactions obtained, the results were classified as: `negative', `traces', 1, 2 and 3, recorded as –, ±, +, ++ and +++, respectively.

**Case Definition:** Mammary glands which had no detectable abnormalities, but had positive CMT and were bacteriologically positive.

**Statistical Analysis:** All statistical analysis was performed using SPSS software (version 11). The somatic cell counts were analysed by ANOVA with animal age and species.

## RESULTS

**Period Prevalence of Subclinical Mastitis:** During the study period, 260 milk sample were collected from 130 ewes. Positive CMT results were recorded from 34 (17%) mammary samples. Of all the milk samples examined, bacteria were isolated from 24 (9.23%) ewes. Of the 34 CMT positive and the 24 bacteriologically positive milk samples, 17 were both CMT and bacteriologically positive (Table 1). The specificity and sensitivity of CMT test in detecting subclinical mastitis were 92.1 and 75%, respectively (Table 1). The $\hat{e}$ value of demonstrated agreement between the CMT results and culture test.

According to the definition of subclinical mastitis (Section 2), there were 17 (18.3%) ewes affected during the lactation period.

**Effect of Age on the Occurrence of Subclinical Mastitis:** A significant ($P < 0.05$) relationship was detected between age and period prevalence of subclinical mastitis in each flock (Fig. 2). Subclinical mastitis occurred more frequently in old (>2 years old) and multiparous ewes than in young (2 years) and primiparous ewes.

Table 1: The relationship between bacteriological and CMT results of milk sample

| | | Bacteriology | | |
|---|---|---|---|---|
| | | + | - | Total |
| CMT | + | 17 | 15 | 32 |
| | - | 8 | 218 | 226 |
| Total | | 24 | 236 | 260 |

$\kappa$ Value: 0.1; specificity: 94.02%; sensitivity: 75%; proportion positive by CMT: 17%; proportion positive by culture: 9.23%

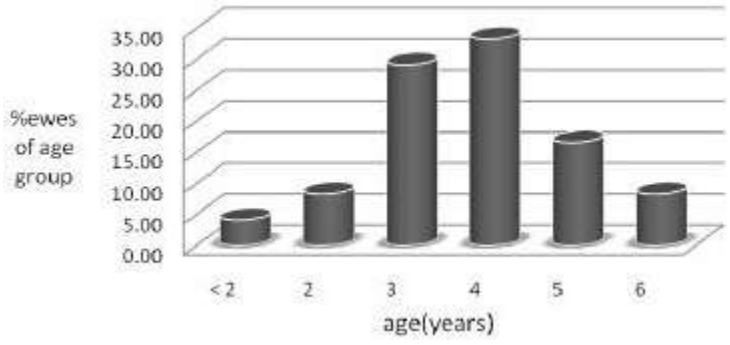


Fig. 2: The percentage of ewes with positive CMT at each age group

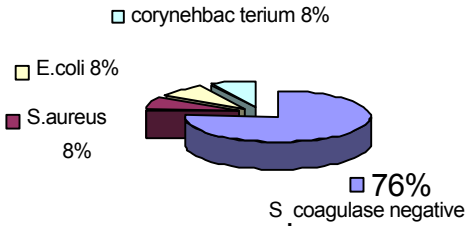


Fig. 3: Bacterial isolates associated with a positive CMT

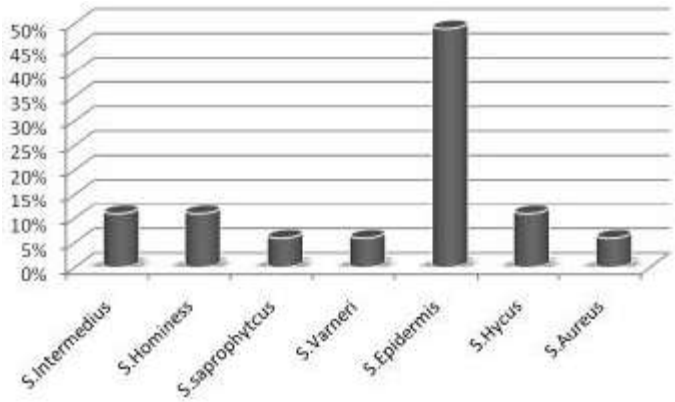


Fig. 4: Percentages of species identified from subclinical staphylococcal intramammary infection in ewes

**Bacterial Isolates:** Distributions of microbial isolates responsible for subclinical udder infection were: coagulase negative staphylococci (76% of isolates), *E.coli* (8%), *Staphylococcus aureus* (8%) and *Corynehbacterium* spp. (8%) (Fig. 1).

## DISCUSSION

Ovine mastitis is an important disease of sheep, with serious financial consequences. In previous studies, it has been repeatedly confirmed that the teat is the portal of entry of the causal agents [11]. The occurrence of clinical and subclinical mastitis in different breeds of sheep has been investigated in various parts of the world [3]. Inflammation of the mammary gland (mastitis) in sheep is predominantly subclinical [12].

Prevalence of Intra mammary infection (IMI) increased with age in sheep in agreement with other studies [3]. It may be due to increased length of exposure to pathogens in older animals compared to younger animals. Additionally, where the duration of infection is long and the spontaneous cure rate low, prevalence will increase [13]. As previously reported, Coagulase negative staphylococci are the predominant bacteria causing subclinical mastitis [14]. CNS are common isolates from the respiratory tract, the teat skin, the teat-end as well as from milk [13].

CNS isolations have been associated with elevated somatic cell count and milk yield reduction, increases in concentrations of NAGase, albumin and salt is the consequence of destruction of glandular elements of mammary gland [15, 16]. In our study, CMT test showed 17% subclinical mastitis that it higher than bacteriological culture (9.23%). It's in agreement with [7], respectively 71% compared with 51%. In this study 8% of isolates was *S.aureus*. Intramammary infections caused by *S. aureus* warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis [17]. Bor *et al*. [8] isolated 93% CNS and We isolate 76% CNS. The isolated CNS species in positive samples were *Staphylococcus epidermidis, S. Intermdius, S. Hominesss, S. Saprophytcus, S. Varneri, S. Hycus, S. Aureus*. The most commonly isolated CNS species in persistent subclinical IMI in goats and sheep are *Staphylococcus epidermidis*, *S. caprae*, *S. simulans*, *S. chromogenes* and *S. xylosus* [18- 20]. Incidence of main CNS isolated (*S. epidermidis*) was in agreement with results reported earlier [21, 2]. *S. epidermidis* and *S. simulans* are among the most prevalent causal microorganisms in ewes [17].

It was concluded that, coagulase negative staphylococci was major cause of subclinical mastitis and the higher prevalence of SCM occurred in older ewes.

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