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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Study of subclinical mastitis in dairy ewes of the Sarab city, Iran

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Abstract

Mastitis, inflammation of the mammary gland, is one of the most common reasons for culling ewes in commercial sheep flocks. Mastitis is usually due to a bacterial infection. To investigate the periodic prevalence, etiology and some epidemiological features of subclinical mastitis in ewes from the Sarab region, milk samples from 160 lactating ewes were aseptically collected for bacterial examination. An association was observed between the occurrence of subclinical mastitis and the age of ewe. The periodic prevalence rate of SCM was 7.5%. Staphylococci were the most prevalent bacteria, representing 83% of the isolates. Coagulase-negative staphylococci (CNS) (71%), was the most prevalent species followed by *Staphylococcus aureus* (12%), *Escherichia coli* (9%) and *Corynebacterium* (8%) of the isolates. In conclusion, subclinical mastitis seemed to be an important health for milking sheep in the Sarab region of Iran.

Keywords: Subclinical mastitis; ewes; Sarab; Iran

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Introduction

Mastitis is defined as inflammation of the mammary glands. Inflammation is most commonly due to infection (intramammary infection or IMI) but may also be due to injury and less commonly due to allergy and neoplasm (Las Heras et al., 1999). If inflammation is present without a detectable IMI, it may be due to udder injury (Mavrogenis et al., 1995; Al-Majali and Jawabreh, 2003). The definition of subclinical mastitis is an inflammation that is not readily detected clinically but adversely affects production (Batavani et al., 2003). What constitutes subclinical mastitis in sheep in terms of level of somatic cell counts (SCC), California mastitis test (CMT) and bacteriological culture results are not as clearly defined as for dairy cattle (Gougoulis et al., 2007). Although subclinical mastitis occurs worldwide, its economical importance is especially significant in the Mediterranean countries, because these are the highest in sheep milk producers within the EU (Batavani et al., 2003). Few researchers have studied the incidence and the consequences of the subclinical form of the disease (Jones, 1991). The objective of this investigation was to determine the prevalence and etiology of subclinical mastitis and its relationship with CMT in sheep.

Materials and Methods

This study involves milk samples from 320 ewes belonged to 15 flocks, selected by stratified random sampling, in Sarab city in west Azarbaijan 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. Samples were collected between June and July of 2011. Udders and mammary secretions were examined for macroscopic signs of abnormality. Mastitis that develops during the dry period or late lactation is often not noticed until the subsequent lambing as well as, in the case of non-dairy sheep, at shearing time. 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 discard of the first three streams of milk. Samples were kept at 4°C during

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transportation to the laboratory for bacteriological analysis which was carried out 2h after collection. All milk samples require bacterial culture were mixed well and a standard loop full (0.01 ml) from each milk sample was inoculated on the surface of blood agar containing 5% of washed sheep red blood cells and MaConkey 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 six or more bacterial colonies of the same type on the medium was considered to be significant and the samples were 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) as described by Quinn et al. (1994). Measuring somatic cell levels in the milk is a surrogate measure of mastitis, i.e., inflammation as a response to infection. Many factors other than infection will elevate SCC. What is most important is what levels of SCC are associated with decreased milk production. The California mastitis test (CMT) was applied to all samples collected using the method of Schalm et al. (1971). According to the reactions obtained, the results were classified as: negative, traces, 1, 2 and 3, recorded as -, ±, ++ and +++, respectively. Mammary glands which had no detectable abnormalities, but had positive CMT and were bacteriologically considered as positive. All statistical analysis was performed using SPSS software.

Results

During the study period, 320 milk samples were collected from 160 ewes. Positive CMT results were recorded from 42(13%) mammary samples. Of all the milk samples examined, bacteria were isolated from 28(7.5%) ewes. Of the 42 CMT positive and the 28 bacteriologically positive milk samples, 20 were both CMT and bacteriologically positive. The specificity and sensitivity of CMT test in detecting subclinical mastitis were 92.1 and 75% respectively. According to the definition of subclinical mastitis there were 21(13%) ewes found affected during the lactation period. A significant ($P<0.05$) relationship was detected between age and period prevalence of subclinical mastitis in each flock (Fig. 1). Subclinical mastitis occurred more frequently in old (>2 years old) and multiparous ewes than in young (2 years) and primiparous ewes. Distributions of microbial isolates responsible for subclinical udder infection were: coagulase negative staphylococci (71% of isolates), *Staphylococcus aureus* (12%), *E. coli* (9%) and *Coryneh bacterium* SPP. (8%) (Fig. 2 & 3).

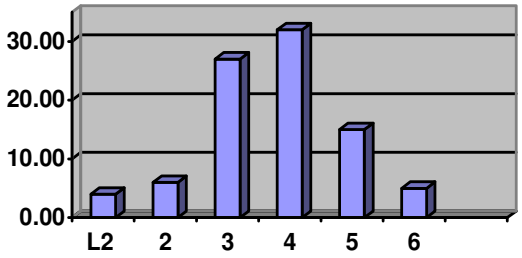

Fig. 1: The percentage of ewes with positive CMT at each age (year) group; L2: below 2 years

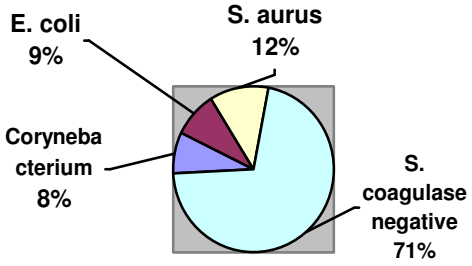

Fig. 2: Bacterial isolates associated with a positive CMT

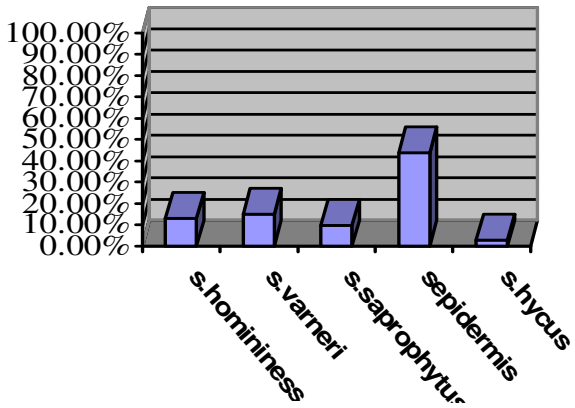

Fig. 3: Percentages of species identified from subclinical staphylococcal intramammary infection in ewes

Discussion

Mastitis is a major problem, estimated to affect ewes per lactation. The clinical manifestation of mastitis can range from sudden gangrenous mastitis with severe illness to chronic mastitis with abscess formation (Contrease et al., 1997). Ovine mastitis is an important disease of sheep with serious financial consequences. In previous studies, it has been reported that teat is the portal of entry of the causal agents (Menzies and Ramanoon, 2001).The occurrence of clinical and subclinical mastitis in different breeds of sheep has been investigated in various parts of the

world (Mavrogenis et al., 1995). Inflammation of the mammary gland (mastitis) in sheep is predominantly subclinical (Quinn et al., 1994). Prevalence of intramammary infection (IMI) increased with age in sheep in agreement with other studies (Al-Majali and Jawabreh, 2003). 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 care rate low, prevalence will increase (Contrease et al., 1997). Previously it has been reported that coagulase negative Staphylococci is the predominant bacteria causing subclinical mastitis (Bergonier et al., 2003). CNS are common isolates from the respiratory tract, the teat skin, the teat-end as well as from milk (Las Heras et al., 1999). 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 (Jones, 1991). In our study CMT test showed 13% subclinical mastitis that it is higher than bacteriological culture (7.5%), and in agreement with Batavani et al. (2003). In this study 12% of isolates was *S. aureus*. Intramammary infections caused by *S. aureus* warrants special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis (Contreras et al., 2007). Bor et al. (1989) isolated 93% CNS and we isolated 71% CNS. The isolated CNS species in positive samples were *Staphylococcus epidermidis*, *S. intermedius*, *S. hominess*, *S. saprophytcus*, *S. varneri*, *S. hycus* and *S. aureus*. The most commonly isolated CNS species in persistent subclinical IMI in goats and sheep are *S. epidermidis*, *S. caprae, S. simulans*, *S. chromogenes* and *S. xylosus* (Bergonier et al., 2003). Incidence of main CNS isolated (*S. epidermidis*) was in agreement with results reported earlier (Las Heras et al., 1999). *S. epidermidis* and *S. simulans* are among the most prevalent causal microorganisms in ewes (Contreras et al., 2007). It was concluded that coagulase negative staphylococci was major cause of subclinical mastitis and the higher prevalence of SCM occurred in older ewes. Because milk out time is very short in small ruminants, udder preparation needs to be done quickly as well, to prevent the loss of the effect of the udder stimulation on let-down. If udders are washed in preparation for milking it is very important that they are dried sufficiently to prevent environmental mastitis, in particular *Pseudomonas aeruginosa* mastitis, due to wet udders. One study compared goat herds that washed and dried udders with individual towels to herds that used a common wash towel and air dried the udder, and found that the latter practice was associated with higher rates of IMI. It may be better to only selectively wash dirty udders or reduce the number of units per milker to allow more time to properly dry udders, to help avoid this problem (Gougoulis et al., 2007).

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