Prevalence and etiology of subclinical mastitis in Buffalo of the Tabriz region, Iran

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Abstract: To investigate the period prevalence, etiology and some epidemiological features of subclinical mastitis in buffaloes from the Tabriz region, milk samples from 51 lactating buffaloes were aseptically collected for bacterial and California mastitis test and somatic cell counte. An association was observed between the occurrence of subclinical mastitis and lactation number of buffaloes. The periodic prevalence rate of SCM was 27.36%. *Staphylococci* were the most prevalent bacteria, representing 48.55% of the isolates. Coagulase-negative staphylococci (CNS) (36.18%), was the most prevalent species followed by *staphylococcus aurous* (14%). *Lactobacillus, Corynebacterium bovis* and *Bacillus subtilis* was the subsequent bacterial groups in importance according with the distribution among flocks representing 14%, 8% and 7% of the isolates. Coagulase-negative Staphylococci were the most prevailing isolates from samples that showed positve CMT results.
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1. Introduction

Mastitis, which is a complex and costly inflammation of the mammary gland, is among the most important diseases in dairy herds (Beheshti et al., 2010). Mastitis occurs in clinical and subclinical form in buffaloes (Sharma and Sindhu., 2007). 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 (Las Heras et al, 1999). The prevalence of this form of disease is 15-40 times more than clinical form and therefore causes high economic losses (Sharif et al., 2007).

Bansal et al., (1995) reported that subclinical mastitis was found in 23.93% of buffaloes and 11.32% of buffalo's udder quarters. Few researchers have studied the incidence and the consequences of the subclinical form of the disease. The importance of subclinical mastitis as a limiting factor in milk production in cows is well documented (Al-Majali et al., 2003). Subclinical mastitis has also adverse effects on the hygienic quality and physicochemical properties of milk (Hamed et al., 1993).

The processing of such milk results in substandard and sub-optimal output of finished fermented products like yogurt and cheese (Sharif et al., 2007). The purpose of present study was to determine the prevalence and etiology of mastitis in Azarbaijan buffaloes.

2. Materials and methods

2.1. Flocks and survey design

Milk samples from 51 buffaloes, selected by stratified random sampling, located in flocks in Tabriz province in northwest of Iran. Within each flock buffaloes were randomly selected and sampled. Identity, Lactation parity and Days in milk were recorded. Abnormalities on the udder were recorded.

2.2. Milk sampling and bacteriological procedures

Samples were collected between November 2010 until March of 2011. Udders and mammary secretions were examined for macroscopic signs of abnormality. Milk samples (20 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 ºC 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., (1994).

2.3. Somatic cell count

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.

2.4. Case definition

Mammary glands which had no detectable abnormalities, but had positive CMT and were bacteriologically positive.

2.5. Statistical analysis

All statistical analysis was performed using SPSS software (version 16). The somatic cell counts were analyzed by ANOVA with animal parturition data and parity.

3. Results

3.1. Period prevalence of subclinical mastitis

During the study period, 201 milk sample were collected from 51 buffaloes. Positive CMT and SCC results were recorded from 70 and 92 (34.82 and 45.77%, represently) milk samples. Of all the milk samples examined, bacteria were isolated from 173 (86.07%) quarters . Of the 70 CMT positive and the 173 bacteriologically positive milk samples, 55 sample were both CMT and bacteriologically positive (Table 1). The specificity and sensitivity of CMT test in detecting subclinical mastitis were 31.79%; and 46.43%, respectively (Table 1). The κ value of demonstrated poor agreement between the CMT results and culture test.

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

		Bacteriology		Total
		+	-	
CMT	+	55	15	70
	-	118	13	131
Total		173	28	201

specificity: 31.79%; sensitivity: 46.43%; proportion positive by CMT: 34.82%; proportion positive by culture: 65.17%.

According to the definition of subclinical mastitis, there were 55 (27.36%) quarters affected during the lactation period.

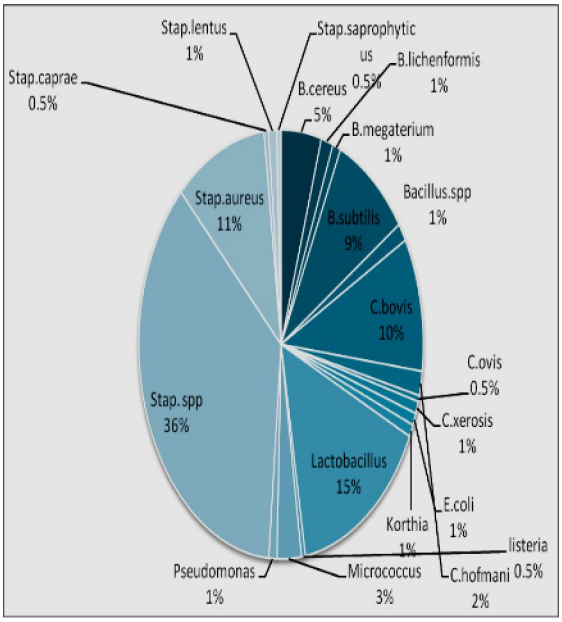

Figure 1: Bacteria isolates

3.2. Effect of lactation stage

The buffaloes in their 3th to 4th month of lactation stage were more susceptible (37.94%) to subclinical mastitis, followed by 1st to two month (31.02%), 5th to 6th month (10.34%), and 7th month of lactation stage (6.89%) (Figure. 3).

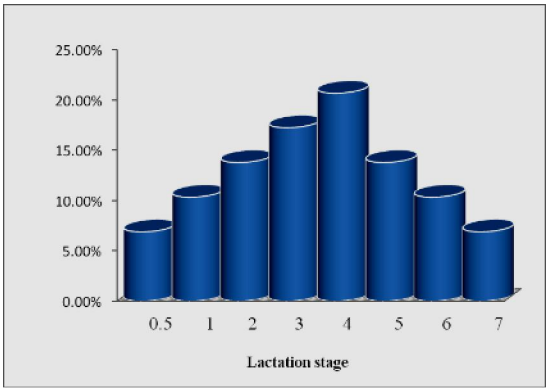

Figure 2. Lactation-stage prevalence of subclinical mastitis

3.3. Bacterial isolates

Distributions of microbial isolates responsible for subclinical udder infection were: coagulase negative staphylococci (38% of isolates); Stap.aurous (14%); Lactobacillus (14%); *Corynehbacterium bovis*

(8%); *B.subtilis* (7%); *E.coli, Pseudomonas, B.cereus, Micrococcus* whichever (3%) and *Listeria, C.xerosis, B.licheniformis, Bacillus.Spp, Korthia, Stap.lentus, Stap.saprophyticus* whichever (1%) (Figure. 4).

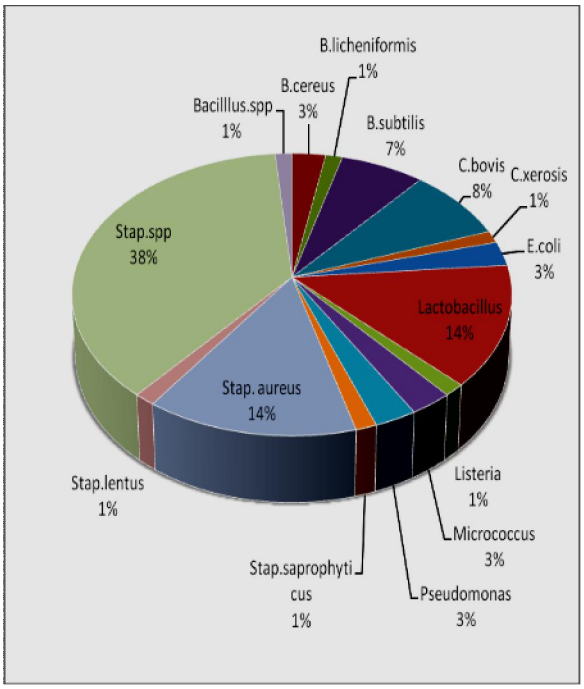

Figure 3. Bacterial isolates associated with a positive CMT

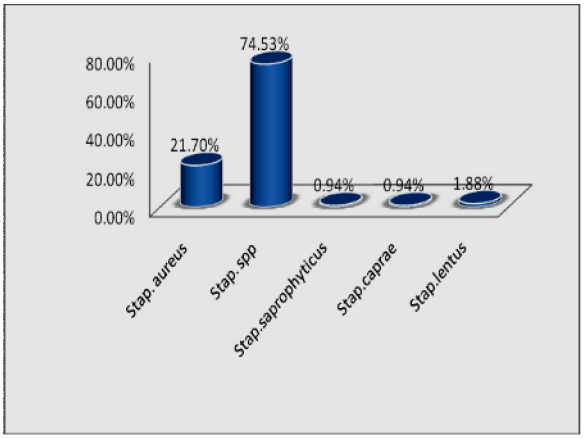

Figure 4. Percentages of species identified from subclinical staphylococcal intramammary infection in buffalo

4. Discussions

Buffalo mastitis is an important disease of this animal, with serious financial consequences (Taraphder *et al.* 2006). In India, the economic loss due to subclinical mastitis was Rs.4,831. Joshi and gokhale reported that the prevalence of subclinical mastitis ranged from 20.72% to 61.73 (Joshi and Gokhale, 2006).

In previous studies, it has been repeatedly confirmed that the teat is the portal of entry of the causal agents (Portolano et al, 2007). In our study, CMT test showed 34.82% subclinical mastitis that it lower than bacteriological culture (45.77%). These finding are in close relation with earlier reports of Dhakal (2006), Ozenc *et al* (2008) and Karimuribo *et al.*, (2006). In this study *Staphylococcus* spp. was predominant mastitogenic organisms (48.6%). Kumar and Sharma (2002) also reported the similar prevalence of Staphylococcus spp (48.94%).

Banerjee (2002) and Sharma and sindhu (2007) observed a higher and lower (54.85% and 38.46%, respectively) incidence of Staphylococcus spp. The prevalence of Coagulase negative staphylococci (CNS) was 38.02% that it was statistically more than coagulase positive ones.

As previously reported , CNS are the predominant bacteria causing subclinical mastitis (Kiossis et al, 2007). CNS are common isolates from the respiratory tract, the teat skin, the teat-end as well as from milk (McDougall et al, 2002). In other animals, 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 (Gougoulis et al, 2007; Maisi et al, 1987). In these cases, 14% of isolates was S.aurous.

Intramammary infections caused by *S. aurous* warrant special attention because this bacterium is responsible for both acute clinical mastitis (gangrenous mastitis) and subclinical mastitis (Contreras et al, 2007). The prevalence of C.pyogenes observed was 8%. Lalrintluanga *et al* (2003) has reported the lower prevalence of C.pyogenes (5.2%).

The results demonstrated that quarter-wise incidence of subclinical mastitis was higher in early lactation phase that it may be due to due to physiological stress of high milk yield and alterations in homeostasis (Rassol et al. 1985). Ronie and Munsterhjelm (1974) reported that The disease occurred most frequently in the 2nd to 3rd month (23.6% of the cases), or 1st week (22.5%) of lactation.

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