

Buffalo Subclinical Mastitis Bacterial Pathogens in Iran

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Abstract. The aim of current study was to determine the bacterial causes of subclinical mastitis in buffaloes of Northwest Iran. 400 buffaloes were tested by California Mastitis Test (CMT) for detection of subclinical cases. 38 (9.5%) quarters were diagnosed as CMT positive. Sterile quarter based milk samples were collected from these quarters and examined for bacterial pathogens. Total Bacterial Count (TBC) and culture were done as described by National Mastitis Council. Hind quarters were most affected (73%) ($p < 0.05$). The most isolated pathogens were the Coagulase Negative *Staphylococci* (CNSs). *Streptococcus* spp. and *Bacillus* spp. were in the next position. Only 2 quarters were affected with *Staphylococcus aureus*. There were no significant differences between the prevalence of isolated bacteria from infected quarters. It is concluded major causes of buffalo's subclinical mastitis are teat skin opportunistic bacteria.

Keywords: Buffalo, Mastitis, Milk, Bacteria

1. Introduction

Mastitis is a major problem in dairy industry because of economic losses. Subclinical mastitis is important due the fact that it is 15 to 40 times more prevalent than the clinical form, because of long duration, difficult to detect, adversely affects on milk quality and production of dairy animals and constitutes reservoir of microorganisms that can affect other animals within the herd due to its contagious nature [1]. Information about the prevalence of mastitis pathogens is of interest especially in treatment and prophylaxis. In previous studies the most isolated bacteria have been CNSs followed by *Corynebacterium* spp. [2, 3, 4, 5 and 6]. In a study the most responsible bacteria for subclinical mastitis in buffaloes was *Staphylococcus aureus* [7]. In Italian buffaloes, 66% of subclinical cases were affected by CNSs [8]. The aim of this study to determine the bacterial causes of subclinical mastitis in water buffaloes of Northwest Iran.

2. Materials and Methods

On the basis of 28% prevalence of subclinical mastitis in Iranian water buffaloes, 400 buffaloes (1600 quarters) were selected for the trial randomly. Buffaloes were housed in tight stalls. The mean age and parity and daily milk yield were 5.5 years old, 3.7 and 4.35 ± 1.3 kg/ml. The buffaloes were clinically healthy with normal milk in appearance. Milking was twice daily in the morning and evening. Sampling for CMT and bacterial culture was done in the morning milking session after discarding two first streams. CMT (Bovi-Vet, Kruuse, Denmark) was done for each quarter foremilk and the results were classified as (-), trace, (1+), (2+) and (3+). Positive quarters were sampled for bacterial evaluations. At first teat ends were washed if contaminated with feces and cleaned with 70% alcohol ethylic for 5 times then 10 ml of milk was collected aseptically from each quarter into a sterile tube. The fresh samples were transferred to laboratory and examined for bacterial count and culture.

TBCs were done according to the standard methods [9] in the microbiology laboratory. Serial dilutions of milk samples were made and 0.1 ml aliquot of each dilution was cultured on Plate Count Agar medium and incubated in 37 °C then colonies were counted after 24 h.

Bacteriological culturing of milk samples was performed according to standards of the National Mastitis

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Council [10]. Ten microliters of each milk sample was spread on blood agar plates (5% defibrinated sheep blood). Plates were incubated aerobically at 37°C and examined after 24 h. Colonies were provisionally identified on the basis of Gram stain, morphology, and hemolysis patterns, and the numbers of each colony type were recorded. Representative colonies were then subcultured on blood agar plates and incubated aerobically at 37°C for 24 h to obtain pure cultures. Catalase and coagulase production was tested for gram-positive cocci. Gram-negative isolates were identified by using colony morphology, gram-staining characteristics, oxidase, and biochemical reactions on MacConkey's agar.

Contagious pathogens *Staph. aureus* and *Streptococcus agalactiae* were considered to cause Intra Mammary Infection (IMI) if at least one colony (≥ 100 cfu/mL) was isolated. For other microorganisms, IMI was defined by the isolation of ≥ 500 cfu/mL and 1 to 3 colony types. Milk samples from which >3 colony types or <500 cfu/mL colonies of any microorganism were isolated were regarded as contaminated or uninfected, respectively.

Statistical analysis was done by SPSS® software (ver. 18).

3. Results

Prevalence of subclinical mastitis on the basis of CMT was 9.5% (38 quarters) (Fig. 1). Hind quarters in comparison with front quarters were mostly affected (73%) and the differences were significant ($p < 0.05$) (Fig. 2). The most isolated pathogens were the Coagulase Negative *Staphylococci* (CNSs). *Streptococcus* spp. and *Bacillus* spp. were in the next position. Only 2 quarters were affected with *Staphylococcus aureus*. There were no significant differences between the prevalence of isolated bacteria from infected quarters ($p > 0.05$).

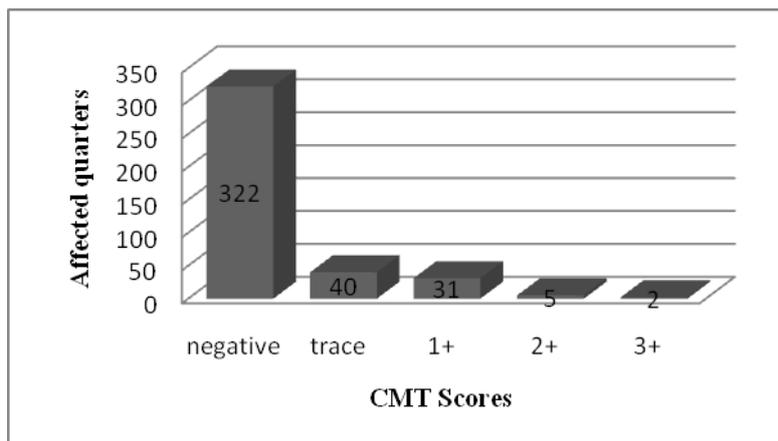


Fig. 1: Prevalence of subclinical mastitis on the basis of CMT

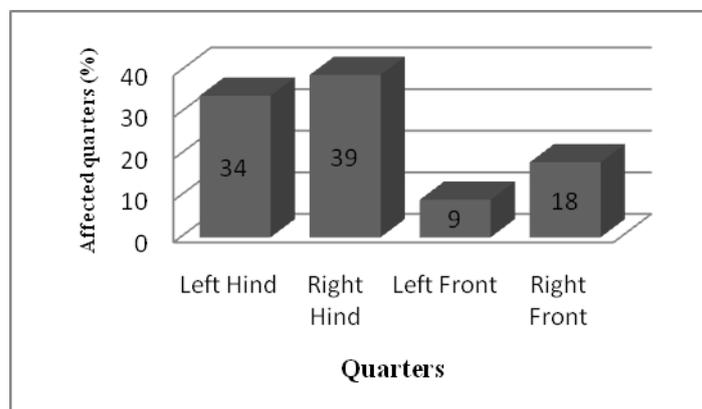


Fig. 2: Prevalence of subclinical mastitis according to quarter's position

Table 1: Isolated Bacteria from quarters affected by subclinical mastitis

Isolated Bacteria	No. Quarters	Percentage
Coagulase Negative <i>Staphylococci</i> (CNSs)	13	34.5
<i>Streptococcus agalactiae</i>	3	8
<i>Streptococcus spp</i>	8	21
<i>Bacillus spp</i>	5	13
<i>Staphylococcus aureus</i>	2	5
Other Bacteria	3	8
Contaminated	3	8
No Bacterial growth	1	2.5
Total	38	100

4. Discussion

One of the most costly diseases in dairy production is mastitis, and even though the buffalo has been traditionally considered less susceptible to mastitis than cattle [11], some researchers have shown similar mastitis frequencies for the 2 species [12, 13 and 14]. Buffaloes have some characteristics that may contribute to greater risk of mastitis. For example, the udder is more pendulous and teats are longer in comparison with cattle. Conversely, buffaloes have a long narrow teat canal, which may be expected to prevent the invasion of microorganisms [8]. In a study in Turkey in 1637 milk samples of Anatolian buffaloes, Ozenc et al. [15] showed that 12.6% of quarters had subclinical mastitis on the basis of CMT. However in a similar study in Pakistan 6.7% of quarters were affected [16]. Alacam et al. [17] reported the distribution of subclinical mastitis as 4.7 to 16.3% in buffaloes, according to CMT results. The prevalence of subclinical mastitis in this study was about 9.5 % that is less than cows and more similar to other studies in buffaloes. It is may be due to anatomical structure of streak canal of buffaloes in comparison to cows or may be due to differences in immunologic defenses. In a survey in Iran [18] prevalence of subclinical mastitis by bacterial evaluations was 13.8%.

The type of bacteria isolated in current study is more like to other studies in other countries [2, 3, 4, 5 and 6]. *Staphylococcus aureus* was not the most important microorganism responsible for subclinical mastitis in buffaloes in this study. In a study in Iran [18] the most prevalent bacteria isolated from subclinical cases were CNSs, *Bacillus spp.* and *Streptococcus spp.*, respectively.

It is concluded major causes of buffalo's subclinical mastitis in Northwest Iran are so like to other buffaloes studied in Turkey, Italy and Pakistan and they are mostly teat skin opportunistic bacteria.

5. References

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