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BACTERIOLOGY OF MILK TAKEN FROM APPARENTLY NORMAL BUFFALO UDDER

Kubra B. Sahi*, Iftikhar Hussain and M. Ashfaque

ABSTRACT

Bacteriological examination and leucocytic counts were conducted on milk samples taken from 150 apparently healthy buffaloes from different areas of Faisalabad. Out of 150 samples 66 (44.00 %) were found to be infected. The pathogenic organisms consisted of staphylococci (45.45%), streptococci (33.57 %), coliform (12.59 %), bacillus (2.10 %), pseudomonas (1.40 %) and yeast cells (0.70 %). The incidence of infection (sub-clinical mastitis) appeared to increase in late lactation. The hind quarters were found more frequently infected than fore quarters. Leucocytic counts with more than 0.1 million cells/ml indicated infection with different microorganisms.

INTRODUCTION

Of the several diseases of buffaloes, udder problem is the most serious as it causes high economic losses by reduction in milk production. Infection of one quarter approximately reduces milk production by 10 to 15 per cent. According to an estimate, average infection reduces the income by 240 million rupees per annum in Punjab (Chaudhry & Khan, 1978), whereas the annual financial loss due to sub-clinical mastitis in India was estimated to be 5.29 billion rupees (Dhanda & Sethi, 1982).

Udder infection has been reported to be caused by many different micro-organisms including *Staphylococcus aureus, Escherichia coli, Corynebacterium bovis, Bacillus cereus, Pasteurella multocida, Mycobacterium tuberculosis,* Streptococcus, Pseudomonas, Klebsiella, Nocardia species, etc.

Leucocytic infiltration in milk gives an indication of infectious changes

Department of Microbiology, University of Agriculture, Faisalabad.

occurring in the udder and is a measure of the quality of milk. The milk from such animals, if consumed, may create zoonotic problems like sore throat and gastrointestinal infections in children (Mian & Jaffery, 1970).

The present study was undertaken to investigate the presence of pathogenic organisms in milk from apparently normal buffaloes and to see their relationship with the development of sub-clinical mastits.

MATERIALS AND METHODS

Five hundred and ninety-seven samples of milk were collected by apparently normal quarters of 150 healthy buffaloes at various stages of lactation from different areas of Faisalabad District. The samples were examined for sub-clinical mastitis by 'indirect' method including leucocytic count and bacteriological examination.

Collection of Milk Samples : Udder and teats were washed and dried before sampling and methylated spirit was applied. Samples were collected in sterilized test tubes after discarding first few strips of milk. The samples were subjected to the following tests :

1. *Leucocyte Count*: After shaking the sample a measured drop of milk (0.01ml) was spread over an area of 2 x 2 cm on a glass slide. The smear was then dried in air and fat was dissolved with xylol and the smear was stained with Wright's stain. Afterwards the slide was dipped in buffer solution for 5-7 minutes to dilute the stain and was then washed in running tap-water. Leucocytic count of the milk was made according to the method introduced by Doxey (1971). The average number of cells per field were multiplied with 2886 (microscopic factor) which gave the total number of cells in 0.01 of milk (0.01 ml milk was spread onto 2 x 2 cm area). Then the figure was multiplied with 100 so as to get the total number of cells/ml of milk.

2. *Isolation of Bacteria* : About 10 ml of fresh milk from each sample was centrifuged at 1000 rpm and a loopful of the sediment was inoculated by streaking method as recommended by Bailey & Scott (1966). For primary culture, blood agar and for pure isolation, selective media were used. The

organisms were identified by observing their colony characteristics, growth pattern on different media, morphology, staining and biochemical characteristics (Schalm *et al.*, 1971).

RESULTS AND DISCUSSION

Of 597 milk samples, 143 (44 per cent) samples were found positive on cultural examination. These findings were in partial agreement with earlier reports of the incidence of sub-clinical mastitis in cow by Kalra (1961), Dhanda & Sethi (1962) and Sharma (1967) ranging from 46 to 60 per cent.

Relative percentage of incidence of different microbial species as found from the present study was: staphylococcus 45.45, streptococcus 34.57, coliform 12.59, corynebacterium 4.20, bacillus 2.10, pseudomonas 1.40 and yeast cells 0.70. Varying incidence of different species of microorganisms has been reported by various workers. Edwards & Smith (1966) observed the incidence of *Staphylococcus aureus*, *Corynebacterium pyogenes* and *Escherichia coli* to be 43.0, 42.0, 4.5 and 2.5 per cent, respectively. Bhatnagar and Mehorta (1969) recorded the incidence of staphylococci to be 50.9 per cent, streptococci 13.2 per cent and pseudomonas 11.3 per cent in mastitic cattle. Jaffery & Rizvi (1975) found that in buffaloes mastitis was due to *Staphylococcus aureus* in 30 per cent, *Streptococcus agalactiae* in 8.7 per cent, *Streptococcus dysgalactiae* in 12.4 per cent, coliform in 51.0 per cent, *Corynebacterium pyogenes* in 3.2 per cent and yeast cells in 0.4 per cent cases. Hashmi *et al.* (1980) observed that staphylococci, streptococci, coliform, corynebacterium, pseudomonas and yeast cells were found as causative agents of sub-clinical mastitis in 43.07, 36.82, 12. 55 4.18, 2.70 and 0.44 per cent experimental buffaloes, respectively. The results of the present study and those of certain others indicated varying incidence of microbial species detected in apparently normal buffaloes and cows udder.

This, however, could be safely stated that staphylococci and streptococci were the most important causative organisms of sub-clinical mastitis. The difference in the incidence of various pathogens could be due to different hygienic and geographical conditions and also due to regular check up of the animal and administration of various broad spectrum antibiotics for the treatment of diseases other than mastitis.

The occurrence of sub-clinical mastitis tended to increase with increased lactation number. Similar observations were reported by Oliver *et al* (1956), Kalra & Singh (1961) and Singh & Baxi (1980) which could be due to low resistance of animals in advanced lactation.

In most of the animals observed for this study, only one quarter was found infected which was in agreement with that of Singh & Baxi (1980). It could be due to the fact that pathogenic organisms might not have entered in all the quarters at the same time as the predisposing factors like teat injury, defective sphincter, etc., could vary from quarter to quarter in an animal. In the present study, it was also observed that the incidence was higher in hind-quarters as compared to fore-quarters. Singh & Baxi (1980) also reported similar findings. The reasons for higher incidence in hind-quarters could be their increased exposure to excreta. Also, during milking they are pulled forward and sideways which may lead to undue stress on them.

Milk samples having less than 100,000 leucocyte cells/ml did not show bacterial growth and were considered as normal (Schalm & Lasmanis, 1968). In the present study the agreement between leucytic count and cultural examination was of the order of 79.44 per cent. Bhatnagar & Mehorta (1969) also reported an agreement of the same magnitude between leucocyte count and cultural examination of milk samples. The percentage of agreement between leucocyte count and bacteriological examination ranged from 72 to 80 (Singh & Baxi, 1980).

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