BACTERIOLOGY OF SUB CLINICAL MASTITIS AND ANTIBIOGRAM OF ISOLATES RECOVERED FROM CROSS BRED COWS

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ABSTRACT
A total of 178 milk samples from apparently healthy lactating crossbred cows (98 Jersey cross and 80 Holstein Friesian cross) were screened for sub-clinical mastitis. The physical examination of sub clinical mastitic milk revealed no changes in colour, consistency and odour. The chemical examination revealed increased pH, somatic cell count and chloride content and positive scores on modified california mastitis test. Out of 98 Jersey crosses and 80 Holstein Friesian crosses which were subjected to examination 30 Jersey crosses(30.61%) and Holstein Friesian crosses 27(33.75%) were found to be affected with subclinical mastitis with a cumulative incidence of 32.02% in cross bred cows. The isolated pathogens from sub-clinical cases and their relative frequencies were: *Staphylococcus* sp.(38.59%), *Streptococcus* sp. (35.08%), *Escherichia coli* (12.28%), *Corynebacterium pyogenes* (8.77%) and *Bacillus* sp.(5.26%). The *in vitro* antibiogram revealed Ceftriaxone(92.66%), Enrofloxacin(89.83%), Kanamycin (88.13%), Ciprofloxacin (82.3%) were most effective, Gentamicin (63.16%) and Chloramphenicol (55.93%) were moderately effective and Tetracycline (32.20%), Ampicillin(30.50%) and Erythromycin (28.81%) were the least effective drugs.

Key words: Sub clinical mastitis, Etiology, Chloride, Somatic cell count, Antibiogram.

INTRODUCTION
Mastitis is the most devastating disease confronting the dairy industry throughout the world but the situation in India is particularly very alarming and requires great attention for its control because of significant loss to dairy producers because of high morbidity, discarded milk, treatment, costs and reduced milk production (Dobbins, 1977). sub-clinical mastitis is a condition in which there is no detectable inflammatory change in the udder and no observable abnormalities in the milk. Often it is more prevalent than the clinical form, it usually precedes the clinical form, it reduces milk production and adversely affects milk quality. Subclinical mastitis is 3 to 4 times more common than clinical mastitis and causes great losses in the dairy herds (Jasper et al., 1982 and Joshi and Gokhale, 2004). The reduction in milk production attributed to sub-clinical mastitis may account for 70%-80% of the total losses (Philpot and Nickerson, 1991). The prevalence of subclinical mastitis has increased enormously in India in the recent years (Tiwari and Sisodia, 2000). To minimize economic losses due to wide prevalence, its early detection becomes most important (Kitchen, 1981).

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In addition to causing massive economic losses to the farmers, the disease is important from consumers and milk processors point of view. This is because the milk from affected animal may harbor the organisms potentially pathogenic for humans and processing of such milk results in suboptimal output of substandard finished fermented products like yogurt, cheese, etc., (Muhammad et al., 1995).

The objectives of the study were to find out the epidemiology of clinical mastitis, to ascertain the physical and chemical changes in milk, to isolate the causative bacterial pathogens from milk and to carry out in vitro antibiotic sensitivity of isolates.

The present study was carried out in Vazhapady taluk of Salem district, which is the highest milk producing district in the state of Tamilnadu of India. It is located between 11° 14’ North latitude and 12° 53’ and between 77° 44’ and 78° 50’ East longitude. It has a population of 593847 cattle and 176521 buffaloes.

**MATERIAL AND METHODS**

Twenty five millilitres of milk was collected aseptically in sterile vials from 178 cows from various mini dairy units, after washing the udder with 1:1000 potassium permanganate and brought to the laboratory to carry out following examinations.

The pH of the milk was estimated immediately after collection with the help of Electronic pH meter. Somatic cell counting was performed by Levowitz - weber modification of the Newman-Lampert stain (Schalm et al., 1971). The chloride estimation were done as per BIS:SP:18 (Part XI) 1981 respectively. Modified California Mastitis Test (MCMT) score were conducted as per Sharma and Rajani(1969).

Milk samples were inoculated with sterile inoculating loop into Nutrient agar, Mac Conkey agar, Mannitol salt agar, Edwards medium and Muller Hinton agar then incubated at 37 °C and checked for growth at 24 hours and 48 hours. Bacterial identification was carried out by studying morphology, culture characteristics and Gram’s staining method (Quinn et al., 2002). A standared disc diffusion method (Bauer et al.,1966) was employed for carrying out Antibiotic sensitivity test using 9 antibiotics viz., Amicillin(10mcg) Ceftriaxone(30mcg), Ciprofloxacin(5mcg), Chloramphenicol(30mcg), Enrofloxacin(19mcg), Erythromycin(15mcg), Gentamicin(10mcg), Kanamycin(30mcg) and Tetracycline(30mcg) (Hi media).

**RESULTS AND DISCUSSION**

In the present study, the cows and buffaloes affected with subclinical mastitis did not exhibit any clinical signs except reduced milk yield. Schalm et al. (1971), Radostitis et al.(2000) and Chakarbarti (2004) stated that there were no clinical signs associated with subclinical mastitis as well as no physical abnormalities in the milk. Similar observations were made in the present study.

The physical examination revealed that the color, consistency and odour of milk were found to be normal. Saravanan (1997) observed that the color, consistency and odour of milk from cattle affected with sub clinical mastitis were normal.

The range of pH value of the apparently healthy cows were from 6.44 ± 0.38 to 6.56± 0.42 which is with in the normal range of pH 6.4 to 6.8 as reported by Vijaykumar (2003). Cows affected with sub clinical mastitis were from 7.28± 0.15 to 7.58 ± 0.35 with a mean value of 7.44 ± 0.35 which itself indicated the likelihood of sub clinical mastitis.

The range of SCC in the samples of sub clinical mastitic milk examined were between 272.58 ± 19.46 x 10⁴ / ml to 298.14 ± 13.92x10⁴ / ml with a mean value of 280.37 ± 2.16. It is well above the normal range of 2x10⁴ to 5x10⁵ as suggested by
Schalm et al. (1971). A cell count of more than 500x10^3 cells/ml of milk was considered to be a positive indication of mastitis (Narayana and Iya, 1954 and Sheldrake and Hoare, 1981).

The mean chloride content of apparently healthy cows was 0.12 ± 0.05g%, which is in the normal range of 0.08 to 0.14g% as reported by Patil (2001). However, samples suspected for subclinical mastitis had a mean chloride content of 0.20 ± 0.05g%. Schalm et al. (1971) reported that bacterial infection of udder leads to opening up of the alveolar junction and an increased permeability of capillaries. Sodium and chloride which were higher in extracellular fluid poured into lumen of alveolus. Reneau (1986) opined that the mammary gland infection is the most important factor affecting somatic cell count in milk in sub-clinical mastitis.

A score of +1, +2 and +3 in MCMT was noticed 20.34 %, 66.10 %, 13.56% of quarters in cows affected with subclinical mastitis respectively. The present observation is in accordance with Doxey (1983) who reported high MCMT score in animals with subclinical mastitis.

Out of 98 Jersey crosses and 80 Holstein Friesian crosses which were subjected to examination 30 Jersey crosses (30.61%) and Holstein Friesian crosses 27 (33.75%) were found to be affected with subclinical mastitis. So cumulatively the incidence of subclinical mastitis in crossbred cows was 32.02%. Joshi and Gokhale (2004) reported that incidence of subclinical mastitis varied from 10 to 50 per cent in cows and 5 to 20 per cent in buffaloes in improved and peri urban dairy farms in India. Ahlner and Axelsson (2002) reported that the prevalence of subclinical mastitis was found to be high, which was about 42.2% on a cow-basis and 21.8 % on a quarter-basis. The difference in prevalence observed between the reports from different parts and the present study may be due to differences in management and husbandry condition in the area and lack of awareness of farmers to the loss caused by mastitis.

It is inferred from Table 2, that the percentage of bacterial isolates in cows with subclinical mastitis were *Staphylococcus sp.* (38.59%), *Streptococcus sp.* (35.08%), *Escherichia coli* (12.28%), *Corynebacterium pyogenes* (8.77%) and *Bacillus sp.* (5.26%). A high prevalence of *Staphylococcus* sp. followed by *Streptococcus* sp. in subclinical mastitis in cows was reported by Paul et al. (2000), Shrirame et al. (2002) and Martin et al. (2006). Buragohain and Dutta (1998) isolated *Escherichia coli* and *Bacillus sp.* from cows affected with subclinical mastitis. Sharma et al. (2007) opined that the prevalence of subclinical mastitis was highest during third and fourth lactation and at 3 to 9 years of age and among the isolates *Staphylococcus* sp. occupied prime position. Boynukara et al. (2008) examined 480 milk samples of cows with subclinical mastitis and isolated a total of 106 strains of *S.aureus*.
The descending order of sensitivity of various antibiotics in subclinical mastitis affected cows were Ceftriaxone (92.66%), Enrofloxacin (89.83%), Kanamycin (88.13%), Ciprofloxacin (82.3%), Gentamicin (63.16%), Chloramphenicol (55.93%), Tetracycline (32.20%), Ampicillin (30.50%), and Erythromycin (28.81%).

CONCLUSION
The higher prevalence of subclinical mastitis in the lactation period is to be viewed seriously as it causes great economic loss than clinical mastitis. Hence, programs for control of subclinical mastitis need to be planned accordingly around the routine examination of all lactating cows, and consequently early treatment can be applied towards positive cases rapidly for preventing their conversion towards clinical form and ultimately the loss can be reduced.

REFERENCES

