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Research Article

# Studies on comparative evaluation of various screening tests for detection of sub clinical mastitis in cows

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Mastitis is the inflammation of the mammary gland. It is disease complex having different causes, different degree of intensity, and variation in duration and residual effects. It brings physical, chemical, microbiological change in milk thus reducing the yield and quality of milk and pathological change in the glandular tissue of the udder.

It is one of the most complex and costly disease of dairy cattle and buffaloes which results in decreased productivity and thus causing considerable economic losses to farmer in dairy industry. The prevalence of sub clinical mastitis (SCM) has increased enormously in India in the recent years because the cow's udder has to undergo rapid changes in relation to size and adjustment for rapid removal of large volume of milk, as such it is prone to injury and infection (Chakrabarti 2005).

Approximately 75% economic losses from sub clinical mastitis are due to loss of milk production. Loss of milk production is more in sub clinical mastitis than in clinical mastitis because of lack of perceptible symptoms of inflammation and no observable physical changes in milk secretion. Detection of sub clinical mastitis is more difficult and it continues to have adverse effects on quality and quantity of milk. The milk of the infected cow is deficient in butter fat and sugar content and is unfit for human consumption. The disease is also important from the public health point of view because the pathogenic organisms may be transmitted to the human especially when consumed raw milk and antibiotic residues in treated milk may affect the human health. Thus a rapid test for detection of sub clinical mastitis would be useful.

## Materials and Methods

A total of 300 quarter milk samples of 78 apparently healthy cows of different lacteal stage were collected aseptically. All the milk samples were subjected to various diagnostic tests like somatic cell count (SCC), electrical conductivity (EC), pH, modified California mastitis test (CMT), White side test (WST) and Trypsin inhibitor activity. To ascertain the comparative sensitivity in relation to direct test i.e. bacteriological examination of milk, the milk samples were collected aseptically. Udder and teat were washed with water and air dried, then each quarter was wiped off by spirit swab. After discarding 10-15 ml of fore milk, 30 ml of fore milk from each teat was collected in sterilized test tube and streaked on blood agar, Nutrient agar and Mac-Conkey lactose agar plates. These Petri dishes were incubated for 24 hours at 37°C. The isolated colonies were fished out and pure culture was obtained. Isolation and identification of bacteria was done using standard procedures of Cowan and Steel (1975).

## Results and Discussion

A total of 300 milk samples from 78 cows were subjected to various indirect and direct tests. The prevalence rate by different tests is shown in the table.

**Table-1 Prevalence of sub clinical mastitis in cows by various diagnostic tests**

| Diagnostic test | Positive animals (out of 78 animals) | Positive quarter (out of 300 quarter) | Efficacy in term of sensitivity |
|---|---|---|---|
| SCC | 55 (70.51) | 139 (46.33) | 100.00 |
| EC | 47 (65.82) | 119 (39.66) | 84.37 |
| CMT | 40 (51.28) | 92 (30.66) | 63.80 |
| Trypsin inhibition | 30 (38.46) | 78 (26.00) | 55.55 |
| WST | 40 (51.28) | 89 (29.66) | 62.74 |
| Milk pH | 30 (38.46) | 64 (21.33) | 29.41 |
| Cultural exam. | 47 (60.25) | 117 (39.00) | - |

Figures in parenthesis indicate the percentage

Prevalence of sub-clinical mastitis according to cultural examination alone was 60.25 per cent (47/78) in cows and 39.00 per cent (117/300) in quarters. With the criterion of SCC > 5 lakh cells/ml of milk, the prevalence of SCM was 60.25 per cent (47/78) in cows and 46.33 per cent (139/300) in quarters.

Quarters showing both SCC (above 500,000 per ml) and culture examination positive were 39.00 per cent (117/300). Only 7.33 per cent (22/300) of the quarter with SCC (above 500,000 per ml) positive were found culturally negative.

Almost similar infection status has been reported by Saxena *et.al.* (1993). They reported 64 per cent cow and 38.7 per cent quarter prevalence of sub-clinical mastitic milk. Saravanan *et al.* (2000) reported 38.17 per cent quarter infection rate. Dutta and Rangnekar (2001) reported high incidence of sub-clinical mastitis (64.4 per cent) by culture examination.

Out of 300 quarter milk samples 117 quarter milk samples were culturally positive and had somatic cell count above 5 lakh cells/ml and only 22 quarter milk samples having somatic cell count above 5 lakh cells/ml were culturally negative. These samples were considered as "non specific mastitis" and failure to detect pathogenic organisms may be due to intermittent excretion of the organisms or their disappearance because of spontaneous recovery (Tolle, 1975). It might be possible that these were having anaerobic organisms, mycoplasma or fungi.

The animal wise prevalence rate of SCM by cultural examination (60.25%) was comparable to SCC (70.51%) and EC (65.82%). However quarter-wise cultural examination of milk showed 39.00% prevalence rate for SCM and was comparable to EC which has detected 39.66% of quarter positive for SCM.

**Table-2 Mean ± SE of SCC and E C in sub-clinical mastitic and normal milk samples**

| Test / Status | SCC | EC |
|---|---|---|
| **SCM** | | |
| **Mean ± SE** | 1.900 ± 0.53 | 6.51 ± 0.21 |
| **Range** | 0.5412 to 8.0479 | 5.9 to 9.3 |
| **Normal** | | |
| **Mean ± S E** | 0.4621 ± 0.09 | 5.31 ± 0.10 |
| **Range** | .2370 to 0.5000 | 4.19 to 5.85 |

Highest prevalence of sub clinical mastitis increased with increase in lactation and reached the peak in 3rd to 6th lactation. This could be due to lowered resistance of the animal as the lactation number increased and improper functioning of the

teat sphincter as mentioned by Singh and Baxi (1980), Saini *et al* (1994), Shinde *et al* (2001) recorded higher prevalence of sub clinical mastitis with increase in lactation number.

In 117 sub-clinical mastitis milk samples SCC were in the range of 0.5412 to 8.0479 million cells/ml of milk with the mean ± SE value as 1.900 ± 0.53 million cells/ml. Remaining 183 samples were considered as normal milk samples as these samples were culturally negative. Mean ± SE value of SCC in these samples were 0.4621 ± 0.09 million cells/ml with the range of 0.2370 to 0.4985 million cells/ml.

Two samples were found positive with culture but electrical conductivity was below 5.9ms/cm whereas four samples had electrical conductivity above 5.9ms/cm while negative, culturally. Linzell and Peaker (1975) reported that electric conductivity of milk is a reflection of its Cl, Na and K contents. Higher ionic strength leads to increased EC and conductivity of fore milk at a single check was a highly accurate method for differentiating between infected and uninfected cows. The present study for detection of sub-clinical mastitis by EC was also based on changes in conductivity of foremilk samples. Davis (1975) suggested that the application of difference in conductivity of milk between individual quarters of a cow as a rapid means of detecting abnormal quarter. There are certain conditions such as illness and estrous which cause rise in EC in milk (Linzell *et al.*, 1974). Milk conductivity was also found to be increased as lactation advanced (Sheldrake *et al.*, 1983).

Saxena *et al.* (2001) reported that differential conductivity or comparison of EC of milk between quarters of same animal improved the ability of this test to detect sub-clinical mastitis in some of the quarters. He also reported that when interpretation was made, the factors like history or any signs of systemic disease, estrous, stage of lactation and recent antibiotic treatment should be kept in mind.

In sub-clinical mastitic milk samples E C were in the range of 5.9 to 9.3 ms/cm with mean ± SE as 6.51 ± 0.21 ms/cm. Remaining 183 samples were considered normal as these were culturally negative. Mean ± SE value of EC in these samples was 5.31 ± 0.10 ms/cm with the range of 4.19 to 5.85 ms/cm. Four samples showed higher EC values; which might be due to some other factors like estrous and other systemic diseases. Sheldrake *et al.*, (1983), Oshima, (1978) and Fernando *et al.*, (1981) reported that many other factors such as stage of lactation, parity, herd difference and the amount of fat in milk, also influenced the electrical conductivity of milk.

The efficacy in term of sensitivity will indicate, power of screening test to diagnose a positive case of sub-clinical mastitis, as positive. The sensitivity was highest with somatic cell count (100 per cent), followed by Electrical conductivity (84.37 per cent), California mastitis test (63.80 per cent), White side test (62.74 per cent), Trypsin inhibitor activity (55.55 per cent), pH (29.36 per cent).

The Somatic cell count and electrical conductivity have highest sensitivity, being able to diagnose the case of sub-clinical mastitis. The somatic cell count cannot be used as a screening test under field condition, as it is cumbersome and requires laboratory facilities. Hand held electrical conductivity test is a good screening test under field condition. Suspected cases should be confirmed by cultural examination.

Tijare *et al.* (1999) analysed various indirect tests i.e. BTB card, EC, MCMT and MWST in comparison to culture examination. They reported 39.20, 92.3, 67.2 and 70.40 per cent efficacy with BTB card, EC, MCMT and MWST respectively. Electrical conductivity test was found to be most efficacious and BTB card showed poor correlation.

The electrical conductivity test appears to be the best screening test under field condition for the diagnosis of sub clinical mastitis. Now a days hand held battery operated electrical conductivity meter is available. Farmer can conduct this test simply by dipping the conductivity cell into milk and reading are displayed direct on screen. With this test we can monitor the cows daily and can identify the infected quarter at the early stage. However, when interpretation is based on this single test, the factors like fever, estrous, stage of lactation

and recent antibiotic treatment should be kept in mind.

The CMT is cheaper and quicker but requires special collection of milk and skilled operator. Drawback of this test is that the cow in the first week after calving or in the last stage of lactation gives false positive reaction.

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