



COMPARISON OF ELECTRONIC DETECTOR AND SOMATIC CELL COUNTER IN EARLY DETECTION OF SUBCLINICAL MASTITIS IN CROSSBRED CATTLE

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Received: 15.10.2013
Accepted: 21.12.2013

Abstract

Comparison of Electronic Detector (EC) and Somatic Cell Counter (SCC) in detection of subclinical mastitis, keeping California Mastitis Test (CMT) as standard in crossbred cattle was done. The sensitivity, specificity, Positive Predictive Value (PPV) and Non Predictive Value (NPV) were calculated. Kappa value of EC was higher than that of SCC.

Keywords: *California mastitis Test, Electronic Detector, somatic cell count, subclinical mastitis*

Bovine mastitis remains a complex disease affecting dairy industry and its management is an increasing challenge during last 30 years (Awale *et al.*, 2012). Economic damage due to subclinical mastitis has been mainly attributed to the fact that a subclinically infected cow is a constant source of infection to other cows and to milk production loss (Swinkels *et al.*, 2005). This disease also poses the risk for the transmission of certain zoonotic diseases like Tuberculosis, Brucellosis, Leptospirosis and Streptococcal sore throat to human beings (Radostits *et al.*, 2000).

Subclinical mastitis is subtle and more difficult to detect affecting the quality and

quantity of milk without any clinical signs of illness. It brings more economic loss to farmers than clinical mastitis. Early diagnosis of mastitis is important because it can save not only money but also time and health status of the animal. Various diagnostic techniques based on cultural isolation of organisms, physical and chemical changes of milk have been practiced in India.

The simplest technique which do not require any trained personals or laboratory equipments, is the use of detergent solutions to diagnose mastitis which produce change in the surface tension, structure and conductivity of cell membrane, disturb the osmotic balance and cause cell rupture. The nuclear material that comes in contact with the detergent increases the viscosity (Middleton *et al.*, 2004) resulting in the formation of flakes. CMT is a detergent based on this principle which is the easiest cow side test and most of the farmers are equipped with this, and hence taken as golden standard test in this experiment. It is the reliable, inexpensive, rapid screening test for subclinical mastitis based on the amount of nuclear protein present in the cow milk sample. Increased ion concentration (Na^+ and Cl^-) in the mastitis milk increases its electrical conductivity (Kitchen, 1981). The principle behind automatic electronic detector is that there will be reduced

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electrical resistance when ionic concentration of the mastitis milk increases. The principle of optical fluorescence in staining the nuclear DNA was made use in Delaval automatic somatic cell counter which is a very rapid technique but expensive.

The objective of the present study was to detect subclinical mastitis by the three methods aforementioned and to make a comparative study between them.

Materials and Methods

A total of 100 crossbred cattle maintained at the University Livestock Farm, Mannuthy, which were apparently mastitis free were selected for the study. The evening milk samples were collected and used for the detection. CMT and electrical conductivity test were done as cow side tests and SCC was done at the research laboratory using DeLaval counter.

CMT

CMT is used on farms to identify subclinical mastitis by an indirect estimation of somatic cells in milk (Leach *et al.*, 2008). Foremilk from all the four quarters were collected to the CMT paddle. Added equal amount of CMT reagent and swirled well. Observed for any flake or clot formation which indicated positive samples. Results were recorded as 0 (negative), + (trace-subclinical), ++ (mild positive), +++ (clinical mastitis) (Sharma, *et al.*, 2010).

Electronic Detector

EC had solely been used for detection of bovine mastitis on phenotypic level at an early stage (Norberg, 2005). The development of subclinical mastitis was accompanied by a rise in level of salt in the milk, which immediately lowers electrical resistance. Milk was collected into the cup of detector to check the infection status of udder.

Interpretation of results

1. Readings above 300 units are given by milk samples of high quality.
2. Readings between 300 and 250 units are given by samples with progressively increasing incidence of subclinical infection.
3. Readings below 250 units are given when there is rapid increase in severity of infection.

Somatic Cell Count by Delaval cell counter

This counter operates on the principle of optical fluorescence, where by propidium iodide is used to stain nuclear DNA to estimate SCC in milk (Viguier *et al.*, 2009). The milk was collected in sterile vial and carried to the research lab where the Delaval cassette was used to count the somatic cell. The average somatic cell count in the animals under study was observed to be 127 cells per microlitre and hence, the score of 150 was fixed for subclinically infected milk.

Statistical Analysis

Mc Nemar's test was carried out to compare the proportion of subclinically infected animals in both SCC and EC with CMT. Measurement of agreement between SCC and EC with CMT was also done by using Cohen's Kappa statistics. The sensitivity, specificity, positive predictive value, negative predictive value were also calculated for comparing the efficacy of the SCC and EC in detecting subclinical mastitis comparing to CMT.

The following diagnostic test characteristics were determined using CMT as gold standard control to test the effectiveness of EC and SCC over CMT in detection of subclinical mastitis.

$$\text{Sensitivity} = \frac{TP}{(TP+FN)} \times 100, \quad \text{Specificity} = \frac{TN}{(TP+TN)} \times 100$$

$$\text{Positive Predictive Value} = \frac{TP}{(TP+FP)} \times 100,$$

$$\text{Negative Predictive Value} = \frac{TN}{(FN+TN)} \times 100$$

where TP- true positive, FP- false positive, TN- true negative, FN- false negative.

Results and Discussion

Out of 100 crossbred cattle screened for subclinical mastitis, 14 showed CMT+ test, and 14 showed positive in electrical conductivity test. Increased number of somatic cells were noticed in 13 of the samples using Delaval counter. The accuracy of EC and SCC was 96% and 93% (Table 1).

The positive and negative predictive values and likelihood ratios were given in Table 2. In the present investigation, the sensitivity and specificity of the EC were higher than that of SCC. It is accepted that kappa values <0.4 indicate poor agreement, values between 0.4 and 0.75: fair to good agreement and values >0.75: excellent agreement. The kappa value

Table 1. Percentage accuracy of indirect tests used for detection of subclinical mastitis

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy
EC	100	14	12	2	84	2	96%
SCC	100	13	11	4	82	3	93%

TP- True positive, FP-False positive, TN- True negative, FN- False negative

Table 2. Agreement and coefficient of determination of indirect tests used in detection of subclinical mastitis with CMT

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV	PLR	NLR	K
EC	85.71	97.67	85.71	97.67	36.78	0.15	0.834
SCC	78.57	95.35	73.33	96.47	16.90	0.22	0.718

PPV-positive predictive value, NPV- negative predictive value, PLR- positive likelihood ratio, NLR- negative likelihood ratio, K-Kappa (measure of agreement)

of EC is 0.834 and that for SCC is 0.718. This shows that EC exhibited excellent agreement with CMT while SCC had a fair agreement.

Continuous monitoring of the udder health and its careful management is essential for the well being of dairy industry without sufficient economic loss. This can be done by the detection of udder inflammation at an early stage and its manipulation.

In the present study EC was found to be more specific and sensitive than SCC. The SCC is increased above the normal range in case of inflammation or udder infections. The positive reaction of CMT seemed to depend upon the concentration of somatic cells in milk (Sharma *et al.*, 2008). SCC needs adequate laboratory facilities and money, hence CMT and EC were developed as alternatives. Present findings are contradicted with what Sharma *et al.* (2008) had reported regarding SCC. EC had been used as an early indicator of subclinical mastitis in which there will be increased ion concentration which may reduce electrical resistance, but not an indicator of infection. Treatment cannot be started merely on the value based on electronic detector.

Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by Modified White Side Test (MWST) (79.94%). CMT could be used in dairy herd monitoring programmes as a screening test to detect fresh cows with intramammary infection caused by major pathogens. Barbosa *et al.* (2002) reported that SCC and CMT were dependent and highly correlated for diagnosis of subclinical mastitis.

Electrical conductivity of milk has been used for diagnosis of subclinical mastitis. The specific conductance of milk reflects the concentration and activity of sodium and chloride ions. Higher values of these ions represents mastitis. The change in concentration is detected by digital detector. Shahid *et al.* (2011) reported increased sensitivity of EC in bovine subclinical mastitis when compared to surf field test, pH detection and NaOH test. Farmers can easily screen out their animals for subclinical mastitis by using electronic detector and can be able to locate the abnormal quarters. This technique gives the result on spot and can help to know the stage of infection.

The increase in ionic concentration status during the beginning of subclinical mastitis even before the presence of somatic cells may have given EC the greater specificity and accuracy than SCC in the present study.

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