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Comparison of two cow side tests for diagnosis of subclinical mastitis in cows

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Abstract
Bovine mastitis remains as the major infectious disease that affects dairy cattle throughout the world and results in considerable economic losses to dairy farmers. The present study was undertaken to screen dairy cattle of two organized farms using two cow side tests such as electrical conductivity test and the widely used California Mastitis Test. A total of 261 Quarter milk samples from 77 cows were screened cow side for subclinical mastitis using California Mastitis Test (CMT) and electrical conductivity meter (EC). Correlation between the CMT score and corresponding conductivity was analyzed and the use of EC as an alternative in screening of subclinical mastitis is discussed.

Keywords: Subclinical mastitis, cattle, electrical conductivity, CMT

Introduction

Mastitis is the inflammation of the mammary gland resulting in physical and chemical changes in milk and pathological condition of the glandular tissue (Matei *et al*., 2009) [7]. It is one of the most important diseases of dairy cattle resulting in heavy economic losses due to reduction in milk yield, milk discard after treatment, cost of treatment and premature culling. In addition to the reduction in milk quality and quantity, it also causes irreversible damage to the udder tissue and less commonly mortality (Radostits *et al*., 2000) [8]. An annual loss in the dairy industry due to mastitis is estimated to be 526 million dollars in India, in which 70% of losses are attributed to subclinical mastitis (Varshney and Naresh, 2004) [12]. The clinical mastitis is characterized by oedema and painful inflammation of the udder and changes in the colour and consistency of milk. In subclinical mastitis, the milk and the udder appears grossly normal without any visible signs of inflammation of the udder. Hence it is difficult to diagnose subclinical mastitis in a herd and requires use of indirect tests like California Mastitis Test (CMT), detection of electrical conductivity, counting of somatic cells or culturing of milk. The sub-clinical mastitis is more serious and accounts for much greater loss to the dairy industry (Singh and Singh, 1994) [11]. The economics involved and reduction in the quality of milk output of the farm makes it a major concern in dairy industry. For effective control of the disease, identification of the affected animals and their treatment or removal from the herd is important. Early diagnosis of mastitis is vital because changes in the udder tissue take place much earlier than they become apparent.

Diagnosis of subclinical mastitis can be done using various quantitative and qualitative laboratory tests. Those tests that can be done in the farm, without much technological requirements are called as cow side tests. The most popular test is the California Mastitis Test (CMT), which involves the estimation of change in the pH of milk and also the increase in the number of somatic cells in the milk following an infection (Bastan *et al.*, 1997) [2]. The test has an advantage that the technological input required is very less and is easy to perform. The short come of the test is that it is a semi-quantitative test and the result is expressed as a relative score and no absolute measurement of pH or the somatic cell count is estimated. This means that though the test is easy to perform, reading of the result and interpretation of the result requires a certain level of expertise and experience by the personnel.

Normal milk has some electrical conductivity due to the electrolytes present in it. But when a cow is subclinically infected, due to inflammatory change, there will be excess leakage of electrolytes into the udder parenchyma, resulting in increased electrolyte concentration in affected milk, which will result in increased conductivity. This is the principle of measuring conductivity of milk for the diagnosis of subclinical mastitis. The average electrical conductivity of milk from infected udder quarters increases, as they increase in Na + and Clion concentration due to damaged mammary epithelium (Bansal *et al*., 2005) [1] Conductivity sensors are being incorporated in many new automated milking systems. The change in electrical conductivity is one of the earliest manifestations associated with new infections making the early detection and recording of possible mastitis cases. The present paper compares the specificity and sensitivity of electrical conductivity to CMT in detecting subclinical mastitis in organized dairy farms.

Materials and Methods

A total of 261 milk samples were collected from quarters of 77 cows from two organized dairy farms and were examined using the CMT and Electrical conductivity meter. Quarters with clinical mastitis were excluded. Electrical Conductivity was measured with a handheld conductometer model “Milk Checker N-4L” (Orient industries, Japan). In order to estimate the conductivity of a quarter, midstream milk was drawn to a receptacle in the equipment and on pressing the test button, conductivity was displayed on the LCD screen of the equipment. After measuring EC the milk was transferred to the test paddle for CMT.

CMT was done according to the method described by Schalm and Noorlander (1957) [10], at cow side, by mixing an equal volume of milk from each quarter with a 1:1000 dilution of 3% sodium lauryl sulphate and bromocresol in wells of a plastic test paddle. As the plate was rotated gently, any color changes or formation of a viscous gel were interpreted. Scores were given within the range zero to three, with zero for no reaction, one for a weak positive, two for a distinct positive, and three for a strong positive.

Results and Discussion

Results of analyzed milk samples are shown in Tables 1. Of the 261 samples, evaluated, 129 milk samples were positive (50.5 per cent) for subclinical mastitis by CMT and 132 were negative. Among the positive samples 26 (9.96 per cent), 20 (7.67 per cent) and 83 (31.80 per cent) samples were scored as +, ++ and +++, respectively. Based on the results of CMT, the samples were allotted to four groups. Mean EC was calculated from the EC values of samples of each group, which were 5.09±0.04, 5.3±0.10, 5.67±0.16, 6.15±0.09 mS/cm respectively.

Table 1: Results of analyzed milk samples are shown

CMT score	0	1	2	3
CMT positive quarters	132	26	20	83
% population	50.5	9.96	7.67	31.8
EC range(mS/cm)	3.9-6.2	4.1-6.4	3.9-6.7	4.1-9.3
EC avg (mS/cm)	5.1	5.3	5.6	6.2
EC standard deviation	0.513	0.536	0.824	1.137

Correlation between CMT and electrical conductivity was estimated by spearman’s rank correlation coefficient method using SPSS17 software. Correlation coefficient was calculated to be 0.554 and was significant at 1% level. Based on the obtained data, the upper and lower bound of each CMT groups were also calculated. The values were 5.07 to 5.18mS/cm for CMT negative milk sample, 5.10 to 5.54 mS/cm for CMT positive (+) samples, 5.32 to 6.02mS/cm for CMT positive (++) milk samples and 5.95 to 6.3mS/cm for CMT (+++) samples. The R^2 value of the regression equation was 0.976 indicating that 97.6% of the validation in the data can be explained by the equation.

During an episode of infection of mammary gland there will be hyperemia and pathological changes in the blood vessel resulting in loss of integrity of capillaries and other finer blood vessels. This may lead to an increased influx of ions like sodium, potassium and chloride in to the udder resulting in increased electrical conductivity of the milk in case of subclinical mastitis. Ilie *et al*. (2010) [4] reported that the mean electrical conductivity was 4.53 mS/cm for normal milk and 6.31 mS/cm for mastitic milk. Estimation of electrical conductivity using this hand held conductivity meter was found to be easier to transport, handle and use as a cow side test for screening for subclinical mastitis. Further, the result can be readily obtained and reading of result and interpretation requires not much technical expertise or experience as compared to conventional CMT (Janzekovic *et al*., 2009) [5].

According to results of the present study, a significant positive correlation exists between CMT scores and electrical conductivity of the same. Similar findings were also reported by Kasikci *et al*. (2012) [6]. In contradictory to this Reibero *et al*. (2016) [9] reported that EC evaluation through the portable EC meter tested, showed a high proportion of results differing from SCC and CMT results. It is also observed that although electrical conductivity has a tendency to rise with the increased CMT score, the magnitude of rise is not that pronounced. This may be explained due to the fact that the ionic changes in milk may be occurring at a later stage of infection. Hillerton and Semmens (1999) [3] suggested that electrical conductivity can be used for prediction of clinical mastitis in herds. Although estimation of EC can be an effective method in detection of subclinical mastitis in organized dairy farms, the efficacy of the test can be still improved by combining it with the tests that involve estimation of somatic cell counts.

Conclusion

Screening for subclinical mastitis in 261 quarter milk samples revealed about 50% of the animals are having subclinical mastitis. Estimation of electrical conductivity was compared with CMT to detect subclinical mastitis. It was found that EC estimation to be easier than CMT as a screening test. There is a good amount of correlation between the two test results and efficacy of EC can be further augmented if the test is used along with other screening methods.

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