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RESEARCH ARTICLE

Comparison of Different Diagnostic Tests in Subclinical Mastitis in Dairy Cattle

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ABSTRACT

The present study was under taken to find out the comparative efficacy of commonly used diagnostic tests for detection of subclinical mastitis in dairy cattle of Andhra Pradesh. Present communication aims to find out the specificity, sensitivity and predictive value of different indirect tests California mastitis test (CMT), electrical conductivity (EC) and somatic cell count (SCC) taking cultural test as standard in sub clinical mastitis affected cattle. Out of 135 quarter milk samples subjected to CMT, EC and SCC taking cultural examination as standard, the per cent accuracies were found to be 73.33, 70.37 and 71.00 respectively. The false positive reactions were more in CMT (24.60%) followed by SCC (23.70%) and EC (20.40%) where as the false negative reactions were highest in EC (34.90%) followed by SCC (31.60%) and CMT (28.60%). The sensitivity, specificity and predictive value of different tests were studied and it was found that EC had the highest specificity (84.84%) and predictive value (79.59%) with lowest sensitivity (56.62%) than compare with the other diagnostic methods for diagnosis of sub clinical mastitis in cattle. Electrical conductivity can be used as the decision criteria to treat or to cull the animals in herds with high prevalence of subclinical mastitis.

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INTRODUCTION

Clinical mastitis is an individual problem which is characterized by the changes in the udder and milk drawn from it, whereas, sub clinical mastitis is herd problem because it constitutes a reservoir of infection which could be transmitted to other animals of herd. It has also been observed that the incidence and the patterns of causative agents markedly differ from place to place, herd to herd, and time to time. Diagnosis of mastitis at sub clinical stage is vital because changes in the udder tissue take place much earlier than they become apparent. SCM also goes unnoticed and remain a depot for spreading infection to the herd mates (Gera *et al.,* 2006). Subclinical mastitis associated with no apparent changes in the udder or milk composition, although microorganism can be isolated by appropriate culture techniques. Cows with subclinical mastitis had decreased milk production by 10 to 20 per cent with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing. The diagnosis of subclinical mastitis (SCM) is problematic since the milk appears normal but usually has an elevated somatic cell count (Forsback *et al.*, 2010).The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the Modified White Side test (MWT), Somatic cell count (SCC), pH, chlorine and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Leslie *et al.,* 2002). The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell count (SCC) and microbiological status of the quarter. Bacteriological culture of milk samples is the standard method for identifying mastitis. However, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted

(Sharma *et al.*, 2010). Present research was carried out to compare the commonly used diagnostic tests for detection of subclinical mastitis in cattle.

MATERIALS AND METHODS

Quarter milk samples from the different organized dairy farms and lactating animals from individual holdings came to College Hospital, Sri Venkateswara Veterinary University, Tirupati were subjected to CMT, EC, SCC and cultural test in order to detect SCM and the diagnostic efficiency, sensitivity, specificity and positive predictive value of each test taking cultural test as standard.

The udder and teats were cleaned with clean water and dried with clean towels. The teat orifice and the skin around the teat were wiped with cotton soaked in 70% alcohol. About 10 ml of milk was collected from four individual quarters into four different sterilized containers and labeled as RF, RH, LF, and LH duly following aseptic precautions. The samples were brought to laboratory within 2 hours after collection. A total of 1599 quarter milk samples collected from 417 cows were collected and remaining 69 quarters were blind.

The milk samples were subjected to CMT as per the procedure given by Schalm and Noorlander (1957) using a modified CMT reagent (Sharma and Rajini, 1969). About 2.5 to 3.0 ml of milk was drawn from four quarters into four cups of the plastic paddle (Figure 1). To this, equal volume of CMT reagent was added, and mixed by gentle stirring for 15 to 20 sec. Based on the reaction, the results were graded as – (negative), and positive as follows: + (there is precipitate but no gel formation); ++ (the precipitate thickens and forms gel towards the center of the paddle); +++ (distinct gel that adheres to the bottom of the paddle).

Mastitis Detector measures electrical resistance in all four quarters. The Mastitis Detector's sensors detect and analyze minute changes occurring in the electrical resistance of a cow's milk (Figure 2). Milk from the sub clinical mastitis cattle had the high levels of salt content which had lower resistance. By this phenomena differentiate the good quality and the lower quality milk. The results can be interpreted as readings above 300 units considered as healthy quarters, readings between 250 and 300 units may indicate either subclinical mastitis or healthy quarter and readings below 250 units indicate subclinical inflammation of the quarter or at least great risk of it.

Somatic cells counter (DeLaval) was used for estimation of SCC levels in milk. A small amount of milk was sucked up into the cassette and inserted it into the DCC (Figure 3). The sample result showed clearly as cells/ml milk on the display, just 45 seconds after the cassette is inserted. The test results can be interpreted as, samples containing 0 to 2,00,000 cells per ml considered as negative,2,00,000 to 4,00,000 cells per ml may indicate either subclinical mastitis or healthy quarter, and more than 4,00,000 and above cells per ml may indicate mastitis or subclinical mastitis.

The milk samples which have positive reaction on CMT were subjected to cultural examination for isolation of etiological agents (bacteria). A loop full of milk samples were inoculated into nutrient broth and incubated at 37° C for 24 hours aerobically and then a loop full of broth culture were streaked on nutrient agar plates. Based on morphology and Gram's staining properties, cultures were inoculated into specific/selective media like Mac Conkey, Mannitol salt agar, and Eosin-Methylene blue agar (Reddy *et al.*, 2011; Reddy *et al*., 2014).

Percent sensitivity, specificity and percent accuracy were calculated by the formulae of Thrusfield (2005).

RESULTS

Various grades of CMT reaction obtained on screening of quarter milk samples by CMT. While 1154 quarter milk samples were negative for CMT reaction, 445 were culturally positive, further 247, 152, 46 quarter milk samples were showing, +, ++ and +++ CMT reaction respectively. The mean (± SE) electrical conductivity of milk from infected quarters was 270±30 whereas that of uninfected quarters were 360±40 and there was a significant difference (P) between the mean electrical conductivity of culturally positive and negative quarters from the subclinical mastitis cows. Leukocyte count of 59 (44.06%) quarters belonging to animals showed a count of 3,00,000 cells and above per ml of milk.

A total of 135 quarter samples from 35 animals were examined with different tests and the results were compared. Out of the 135 milk samples screened, 65, 49, 59 and 69 samples were positive by CMT, SCC, EC, and cultural examination respectively (Table 1).

Out of these, 65 (48.14%) quarter samples from 29 animals were positive for CMT, where the incidence was 48.74% and 82.85% respectively quarter wise and animals wise. Similarly in electrical conductivity test, 49 (36.25%) quarters belonging to 19 (54.54%) animals showed an electrical conductivity of 270±30 indicating presence of subclinical mastitis. Somatic cell count showed 59 (44.06%) quarters belonging to animals 23 (65.71%) was suffering with SCM. The cultural examination of the same samples could yield the bacteria from 69 (51.11%) quarters of 28 (80%) animals as an evidence of mastitis infection.

The percent accuracy of various diagnostic tests for the detection of SCM, when cultural test was taken as standard and it was shown in Table 2 and Figure 4. The per cent accuracy of CMT, EC and SCC was 73.33, 70.37 and 71.00 per cent respectively. The false positive reactions were more in CMT (24.60%) followed by SCC (23.70%) and EC test (20.4%) and the false negative reactions were more in EC (34.90%) followed by SCC (31.60%) and CMT (28.60%).

Among these three indirect tests, the CMT was 75.38 per cent; EC was 79.59 per cent; whereas SCC was 76.27 percent true positive when compare with the cultural examination of the milk obtained from the individual cattle suffering with the subclinical mastitis.

The Table 3 & Figure 5 shows that the sensitivity and specificity followed by predictive value of positive test. Among the three tests, CMT (71.01%) had the highest sensitivity, followed by SCC (65.21%) and EC (56.52%). Highest specificity was notice with the EC (84.84%) followed by SCC (78.78%) and CMT (75.75%).

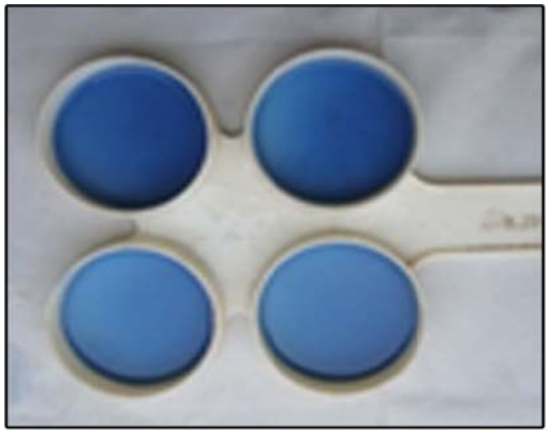

Fig. 1: California mastitis test paddle.

Fig. 2: Mastitis detector (Draminski).

Fig. 3: Somatic cells counter (DeLaval).

DISCUSSION

In SCM, irrespective of the etiological agents involved, the first pathological change noticed is the passage of leukocytes and erythrocytes into the milk as a result of increased permeability of udder capillaries to inflammatory reaction. The intensity of the inflammation can be estimated qualitatively by CMT and quantitatively by SCC. Similarly, the diffusion of sodium and chloride ions into the milk also increases which can be detected by EC. So the presence of the causative agent in the milk can be identified by cultural examination. Thus in the present study four different diagnostic tests namely, CMT, SCC, EC and cultural examination were used for the detection of SCM. The sensitivity and specificity of the above three tests were determined by considering cultural examination

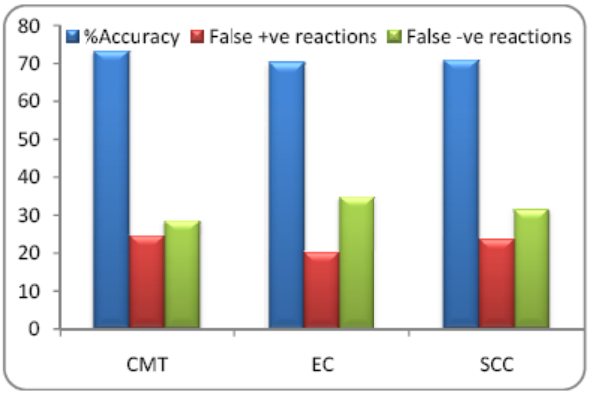

Fig. 4: Percent accuracy, false positive and false negative reactions of various diagnostic tests employed for diagnosis of SCM taking cultural test as standard.

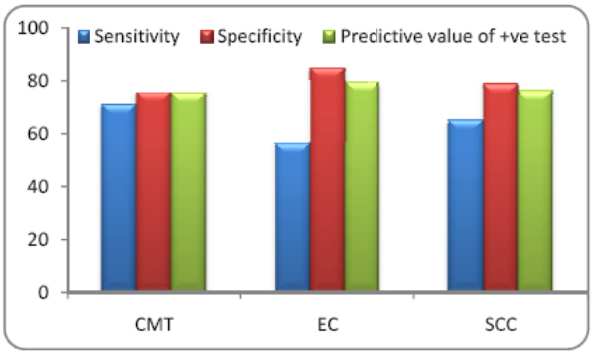

Fig. 5: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural test as standard.

Table 1: Detection of subclinical mastitis using different diagnostic tests

Name of test	Number of quarters		
	Tested	Positive	Prevalence (%)
CMT	135	65	48.14
EC	135	49	36.25
SCC	135	59	44.06
Cultural isolation	135	69	51.11

as standard test. The reasons for variation in the sensitivity and specificity of different diagnostic tests in the present study might be due to the fact that they are designed to detect different types of changes in the sub clinically infected milk.

Sharma *et al*. (2010) compared the sensitivity of the CMT, SLST, and SCC for the detection of SCM in dairy cows and stated that CMT was the most accurate, reliable, diagnostic method after cultural isolation and SCC under field conditions. Siji and Vijaykumar (2007) and Islam *et al.* (2010) reported the better performance of CMT in detecting SCM among the other indirect tests. Sharma *et al.* (2010) reported that the sensitivity of the CMT was 86.07 percent, specificity was 59.70 percent, accuracy was 75.52 percent, positive predictive value was 76.21 percent, and negative predictive value was 74.07 percent, and stated the CMT was the most accurate reliable diagnostic method.

In the present study the SCC of more than 3, 00,000/ ml. of milk was considered as positive for SCM. Sorana *et al.* (2010) reported that the positive diagnosis was

Table 2: Per cent accuracy of various diagnostic tests taking cultural test as standard

Name of the test	Total samples examined	Test positive samples	Test reaction as compared to cultural examination				
			True positive	False positive	True negative	False negative	Percent Accuracy
CMT	135	65	75 (75.38%)	24 (24.60%)	76 (71.40%)	20 (28.60%)	73.33%
EC	135	49	59 (79.59%)	15 (20.40%)	85 (65.10%)	30 (34.90%)	70.37%
SCC	135	59	69 (76.27%)	21 (23.70%)	79 (68.40%)	24 (31.60%)	71.00%
Cultural isolation	135	69	108 (100%)	--	97 (100%)	--	100.00%

(Figures in parenthesis indicates percent)

Table 3: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural test as standard

Name of the test	Sensitivity	Specificity	Predictive value of +ve test
CMT	71.01	75.75	75.38
EC	56.52	84.84	79.59
SCC	65.21	78.78	76.27
Cultural examination	100	100	100

confirmed by the increased number of somatic cells present in milk. The values obtained were between 5, 00,000 and 1.5 million cells/ml in SCM and in healthy cows, somatic cell count has not exceeded the value of 2, 70,000 cells/ml. Sharma *et al.* (2010) reported the sensitivity of the SCC was 88.60 percent; specificity was 97.76 percent; accuracy was 91.94 percent; positive predictive value was 98.33 percent; and negative predictive value was 84.52 percent.

Among the indirect tests the measurement of EC of milk has an advantage over the mastitis detection procedures in that the result is made available immediately without additional effects and large number of animals can be screened by a single visit. EC is considered as sensitive and gives the result on the spot and can help to detect quarter wise prevalence and farmers can easily use this instrument to screen dairy animals for SCM (Muhammad *et al*., (2011).

Jorge *et al*. (2004) opined that EC is better than CMT, whereas Janzekovic *et al.* (2009) claimed that CMT and EC don't exclude themselves mutually complementally to each other. Guven *et al*., (2012) concluded that EC showed similarity with the CMT and the SCC in the detection of SCM furthermore, its reliability would further increase when used together with the other diagnostic methods. Seguya and Mansell *et al.* (2010) compared the efficacy of EC by taking cultural test as a standard test. Gaspardy *et al.* (2012) reported that shortly after calving the EC value generally decreases; however, it was discovered that from the thirteenth week onwards, substantial differences arise between the mastitis and healthy groups of cows and observed a significant ($P<0.001$) increase in EC before the detection of clinical mastitis.

Although, the EC may have same practical advantages in comparison to other diagnostic methods, the predictive value was generally, poor. However, while judging the results of different diagnostic tests of SCM, one should consider the fact that the EC detects the chemical changes, CMT and SCC the cultural examination indicates the presence of bacteria. In essence the cultural examination of milk is very essential as the ultimate aim is detection and prevention of infection.

In the present study per cent accuracy of various (CMT, EC, SCC) tests taking cultural test as standard is summarized that electrical conductivity test has high specificity (84.84%) and predictive value (79.59%) with lowest sensitivity (56.52%) than compare with other diagnostic tests. Though the sensitivity is low, a high specificity and predictive value of the positive test in the present study enables EC to be used as the decision criteria to treat or to cull the animals in herds with high prevalence of sub clinical mastitis in dairy.

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