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Diagnosis of Subclinical Mastitis in Cross Bred Cattle

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Abstract

Subclinical mastitis was diagnosed in 1599 quarter milk samples from 417 apparently healthy lactating cows in and around Tirupati. Diagnosis of subclinical mastitis was done by California Mastitis Test and Electrical Conductivity Test. The quarter-wise incidence of SCM as detected by CMT and EC was 27.83 and 19.95% respectively whereas the animal-wise incidence was 52.28 and 38.13% for CMT and EC respectively in the present study.

Keywords: *Cattle, mastitis, CMT, MWT, EC, SCC*

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INTRODUCTION

Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country [1]. The prevalence of subclinical mastitis in dairy herds is often surprising to producers; moreover, sub-clinically infected quarters can develop clinical mastitis and the rate of new infections can be high. Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value, rendering it of low quality and unfit for processing [2]. 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), SCC, pH, chlorine and catalase tests. These tests are preferred to screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results [3].

MATERIALS AND METHODS

Milk samples were collected from apparently healthy lactation cows in and around Tirupati. In the present study, total of 1599 quarter milk samples were collected from 417 cows and remaining 69 quarters were blind. The udder and teats were cleaned with clean water and dried with clean towels. The teat orifice and the skin around the teats 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 h after collection for culture examination and SCC.

The milk samples were subjected to CMT as per the procedure given by Schalm using a modified CMT reagent and Sharma [4, 5]. About 2.5 to 3.0 mL of milk was drawn from four quarters into four cups of the plastic paddle. 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 – (There is no precipitating and no gel formation – 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. Milk from a quarter infected with subclinical mastitis will have increased salt content, which results in lower resistance. So it can easily differentiate between infected and healthy quarter and separate 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 cell counter (De Laval) was used for diagnosis of subclinical mastitis. A small amount of milk was sucked up into the cassette and inserted into the somatic cell counter (DCC). The sample result showed clearly as cells/mL milk on the display, just 45 sec after the cassette is inserted. The test results can be interpreted as samples containing 0 to 200,000 cells per mL considered as negative, 200,000 to 400,000 cells per mL may indicate either subclinical mastitis or healthy quarter, and more than 400,000 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. A loop full of milk samples was inoculated into nutrient broth and incubated at 37 °C for 24 h aerobically and then a loop full of broth culture was 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 [6].

RESULTS

Quarter milk samples from the seven different organized dairy farms and lactating animals from individual holdings that came to Teaching Veterinary Clinical Complex, Tirupati, were subjected to CMT, EC, SCC in order to detect subclinical mastitis. Various grades of CMT reactions were 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 (Table 1). To standardize the mean (±SE) electrical conductivity readings of normal and mastitic milk, 1599 quarter milk samples were subjected to EC.

Results revealed that the mean (±SE) electrical conductivity of milk from infected quarters was 270±30 whereas that of uninfected quarters was 360±40 and there was a significant difference (P) between the mean electrical conductivity of culturally positive and negative quarters from the subclinical mastitic cows. Employing SCC it was possible to identify 65.20% (45/69) as positive quarters out of 69 infected quarters and consequently false-positive and false-negative reactions were 23.70 and 31.60% respectively and the percent accuracy was 71% (Table 2).

Table 1: *Grades of CMT Reaction Exhibited by 1599 Quarter Milk Samples.*

S. No.	CMT reaction grade	Number of quarter showing CMT reaction
1.	-	1154
2.	+	247
3.	++	152
4.	+++	46
	Total	1599

Table 2: *Detection of Subclinical Mastitis Using Different Diagnostic Tests.*

S. No.	Name of test	Number of quarters		
		Tested	Positive	Prevalence (%)
1.	CMT	135	65	48.14
2.	EC	135	49	36.25
3.	SCC	135	59	44.06
4.	Culture isolation	135	69	51.11%

DISCUSSION

Out of the 135 milk samples screened, 65, 49, 59 and 69 samples were positive by CMT, SCC, EC, and cultural examination respectively. CMT is considered as an important diagnostic tool to detect SCM as it is a reliable, rapid, cheap, simple to perform and easy to interpret and can be performed under field condition. Ramachandraiah *et al.* reported that CMT was satisfactory for the diagnosis of subclinical mastitis [1]. Siji and Kumar reported that the accuracy of CMT was 83.50% [7].

Islam *et al.* reported the better performance of CMT in detecting SCM among other indirect tests [8]. Sharma *et al.* reported that the sensitivity of the CMT was 86.07%, specificity was 59.70%, accuracy was 75.52%, positive predictive value was 76.21%, and negative predictive value was 74.07%, and stated the CMT was the most accurate reliable diagnostic method [9]. Aledany *et al.* reported that the California mastitis test was the best one [10]. Rabbani and Samad reported the comparative prevalence of subclinical mastitis by using CMT as 4.31, 7.33 and 7.38% in mild, moderate and severe reaction to CMT [11]. The leukocyte count, used as an indicator of degree of inflammation of udder is the basis for most indirect tests [12]. Estimation of SCC to detect SCM is considered as sensitive [9]. In the present study, the SCC of more than 300,000/mL of milk was considered as positive for SCM. Rupp and Boichard recorded that the animals with lowest initial SCC possess minimum risk to suffer clinical mastitis in the first calving [13].

Sharif *et al.* reported that the range and mean milk SCC was affected by severity of subclinical mastitis [14]. Aiumlamai *et al.* reported that post-milking quarter samples had significantly higher average somatic cell counts from De Laval cell count than pre-milking quarter samples and demonstrated that post-milking quarter sample suitably represented udder health status based on SCC, which should be routinely used to indicate subclinical mastitis problem in dairy cows [15]. Sorana *et al.* reported that the positive diagnosis was confirmed by the increased number of somatic cells present in milk. The values obtained were between 500.000 and 1.5 million cells/mL in SCM and in healthy cows, somatic cell count has not exceeded the value of 270.000 cells/mL [16]. Ioan reported the limits of the number of somatic cells of cows with subclinical mastitis ranged between 500 and 1500 mL SCCx10-3 [17]. Barrett *et al.* and Moroni *et al.* found an SCC of 200,000/mL of milk between negative and positive samples respectively [18, 19]. Sharma *et al.* reported that the sensitivity of the SCC was 88.60%; specificity was 97.76%; accuracy was 91.94%; positive predictive value was 98.33%; and negative predictive value was 84.52% [9].

Among 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 a 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 [20]. Jorge *et al.* opined that EC is better than CMT, whereas Janzekovic *et al.* claimed that CMT and EC do not exclude themselves mutually complementally to each other [21, 22]. Guven *et al.* concluded that EC showed similarity with CMT and the SCC in the detection of SCM; furthermore, its reliability would further increase when used together with other diagnostic methods [23].

The EC reading below 300 was considered as positive for SCM in the present study. Musser *et al.* reported that absolute EC score for cows with subclinical mastitis was significantly higher than that for cows without subclinical mastitis, and absolute EC score was significantly associated with detection of subclinical mastitis [24]. Norberg *et al.* reported that electrical conductivity (EC) of milk is a potential trait in a breeding program where selection for improved udder health and all EC traits increased significantly ($P < 0.001$) when cows were subclinically or clinically infected [25]. Chahar *et al.* reported that threshold value for electrical conductivity to detect subclinical mastitis in cows was

5.9 ms/cm [26]. Seguya *et al.* compared the efficacy of EC by taking cultural test as a standard and stated that this device did not detect the infected quarters as positive [27]. Ilie *et al.* investigated several traits of EC in order to associate them with the health status of the udder and classified as healthy/clinically infected or subclinically infected and reported that all these traits increased significantly when subclinical or clinical infections were present [28]. Gaspardy *et al.* reported that shortly after calving, the EC value generally decreases [29]; however, it was discovered that from the thirteenth week onwards, substantial differences arise between the mastitic 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 some practical advantages in comparison to other diagnostic methods, the predictive value was generally, poor.

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