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California mastitis test (CMT): An onsite test for screening of subclinical mastitis in buffaloes

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Abstract

Mastitis, especially subclinical mastitis is a major issue for milk producing countries like India due to difficulty in its early detection. An array of causative agents are encountered but the most common ones are *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli* and *Mycoplasma* species. The infectious nature of mastitis causing pathogens requires early screening of milk samples. California mastitis test (CMT) is an indirect, on-farm test for this purpose which is constantly being used by many dairy agencies. Subclinical cases are hard to detect as there is no visible systemic sign in udder. The only inspection of subclinical case can be made by fall in milk production. Milk samples from 44 lactating buffaloes were collected and subjected to CMT for screening. It was found that 74 (61.67%) quarter samples were negative for CMT and 46 (38.33%) quarter samples were positive for CMT. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (++) as 24 (52.17%) and the lowest in CMT score (+++) as 11 (17.39%). The CMT score is based on the number of leukocytes in milk. The positive reaction of CMT represents alkalinity owing to rise of inflammatory cells (SCC).

Keywords: Mastitis, quarter, California mastitis test, leukocyte

Introduction

Due to increasing demand of milk day by day for human consumption, it becomes the need of hour to screen the milk and to control mastitis. Several tests are available for this purpose. One such test receiving acceptance by various dairy agencies is an on-farm test, known as California mastitis test (CMT). Now a days, CMT is widely used as a milk grading test in various countries. CMT is considered as one of the oldest test to detect different stages of udder inflammation under field conditions. Schalm and Noorlander (1957) [11] purported CMT as a low-priced, easily-applicable cow-side test that enables the subjective evaluation of the somatic cells present in a milk sample as an assessment of the probability and severity of intramammary infection (IMI).

The ability to detect the presence of an IMI represents the sensitivity of the CMT and it is calculated as the proportion of quarters that had an IMI with a positive CMT. Whereas, the ability to detect quarters that did not have an IMI represents the specificity of the CMT and it is calculated as the proportion of non-infected quarters that had a negative CMT reaction. The conjunction of these 2 test characteristics describe how well the CMT can discriminate between non-infected and infected quarters (Martin *et al.*, 1987; Dingwell *et al.*, 2002) [5, 2].

The CMT is hinged on the number of milk leucocytes as a measure of udder infection (Schalm and Noorlander, 1957) [11]. The CMT reaction is mainly generated by deoxyribonucleic acid originating in cell's nuclei constituting the inflammatory exudates (Carroll and Schalm, 1962) [1]. The analysis of CMT test is based on scoring system as a score of 0 = 0-200,000 leucocytes per milliliter; trace = 150, 000-500, 000; 1 = 400,000-1,500,000; 2 = 800,000-5,000,000; 3 = over 5,000,000 (Master, 1960) [6].

If the CMT score comes 1, it reflects that milk contain less than one million leucocytes per milliliter whereas, a CMT score of 2.2 reflect milk leucocytes more than one million per millimeter (Smith and Schultze, 1965) [12]. In general, the CMT score of 1 to 3 is an indicative of higher cell count count (Leidl *et al.*, 1963) [4].

These days, several commercially available CMT reagents are available. In a recent study conducted by Rust *et al.* (2021) [9], the effectiveness of a detergent based CMT using domestic detergents was compared with the commercially available CMT reagent. The study revealed that domestic detergents are as good as commercially available CMT reagents and can be used as alternative CMT reagents for farmers to assist in cost effective diagnosis of mastitis.

The test reagent in CMT is generally a sodium or potassium salt of long chain fatty acids which act as anionic-surface-active agents i.e. alkyl sulphates, alkyl sulphonates, alkyl arylsulphonates or alkyl arylsulphates (Leach *et al.*, 2008)^[3]. Equal amount of milk sample and test reagent is poured in CMT paddle and gentle agitation results in lysis of somatic cells releasing cellular DNA. The agglutination of DNA makes the sample look like ‘slimy’ or ‘mucoid’ on account of number of cells present in the sample. The gradation can be done on the basis of visible agglutination on an ordinary scale. It gives a qualitative test to measure milk SCC (Moroni *et al.*, 2018)^[7].

Materials and Methods

Sample collection

A total of 120 milk samples were collected from 44 lactating buffaloes from Instructional Dairy Farm situated at Nagla, Pantnagar and subjected to California mastitis test (CMT) on individual quarter’s milk samples. Milk samples were collected after washing the udder with clean water and dried with cotton cloth. The teat ends were swabbed with a cotton wool soaked in 70% ethanol. The samples were collected in the afternoon during milking time.

In the present study, a commercially available California mastitis test kit was used. This test is also known as “WEIZUR TEST” manufactured by Weizur ® India Private Limited, ANAND, Gujarat. The results were interpreted as per the manufacturer’s instructions given inside the kit on the basis of intensity of color change and gel formation (Table 1). WEIZUR Test kit contains a bottle of CMT test Reagent (500 ml) with CMT test paddle and a Dispensing Pump (Figure1

and 2).

The milk was poured from each teat of buffalo into the four shallow cups of CMT paddle. The paddle was then tilted to equalize milk quantities in the cup. The same amount of CMT reagent was poured into each cup. The paddle was rotated clockwise to mix and to observe the color change and gel formation. Normally, the CMT reagent used in Weizur test is red in color. The purplish or dark purplish color indicates infected milk.



Fig 1, 2: WEIZUR® kit for California mastitis test showing results from affected milk sample

Table 1: Observation and interpretation of CMT score

Presence of gel	Color	Score	Interpretation
No thickening of mixture	Grey	0	Normal Milk
Slight thickening of mixture. Thickening may disappear after rotating paddle for 10 seconds.	Light Purple	1	Weak Positive (+)
Distinct thickening of mixture. Thickening may disappear after rotating paddle for 20 seconds	Purple	2	Distinct Positive (++)
Gel formation and surface of the mixture becomes elevated. Central peak remains projected even after the CMT paddle rotation is stopped.	Dark Purple	3	Strong Positive (+++)

Table 2: Result of animals positive for subclinical mastitis by California mastitis test

CMT				
Quarter				Total
RF	RH	LF	LH	
-	-	-	+++	1
-	-	++	-	1
-	+	-	-	1
-	++	-	-	1
-	-	-	++	1
++	-	-	-	1
-	++	-	++	2
-	+	++	++	3
-	-	++	++	2
-	+	-	+	2
-	+++	-	+++	2
+++	-	-	-	1
+	+	-	+++	3
-	++	-	++	2
-	+	++	-	2
-	-	-	++	1
-	-	++	++	2
-	-	-	++	1
-	-	-	++	1
++	+++	-	-	2
-	+	-	-	1

-	-	++	-	1
-	-	+	++	1
-	-	-	+++	1
-	+++	-	-	2
-	-	-	++	1
-	+	++	+	3
+	-	-	-	1
-	++	-	-	1
-	-	+	+	2
05	14	09	18	46

Results and Discussion

Out of 120 quarter milk samples collected from 44 lactating buffaloes, 74 (61.66%) quarter samples were negative for CMT and 46 (38.33%) quarter samples were positive for CMT (Table 2). With a total of 18 (40.90%) dairy buffaloes were positive for CMT and total of 26 (59.09%) dairy buffaloes were negative for CMT. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (++) as 24 (52.17%) and the lowest in CMT score (+++) as 11 (17.39%). The CMT score is based on the number of leukocytes in milk (Prouty, 1934)^[8]. The positive reaction of

CMT indicates alkalinity which arises due to increment of milk somatic cells. In a similar study conducted by Salama and Saad (2016)^[10] on 326 milk samples collected from 90 lactating buffaloes, 122 (37.42%) quarter samples were positive for CMT and 204 (62.58%) quarter samples were negative for CMT. With a total of 39 (43.33%) dairy buffaloes were positive for CMT and total of 51 (56.67%) dairy buffaloes were negative for CMT. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (+) as 53(43.44%) and the lowest in CMT score (+++) as 25(20.49%).

Table 3: CMT based Scoring of examined quarters' s milk samples

Examined Quarters (Sample No.)	Positive Samples		Negative Samples		Score of Positive Quarter Samples					
	No.	%	No.	%	+ (Weak Positive) (1)		++ (Distinct Positive) (2)		+++ (Strong Positive) (3)	
					No.	%	No.	%	No.	%
120	46	38.33	74	61.67	14	30.43	24	52.17	08	17.39

Table 4: Results of examined animals on CMT

Parameters	Examined Animals (No.)	Percentage (%)
CMT positive	18	40.9%
CMT negative	26	59.1%
Total	44	100%

Conclusion

From the present study, it can be concluded that incidence of subclinical mastitis per quarter's milk samples and per animal was 46 (38.33%) and 18 buffaloes (40.9%) respectively (Table 3 and 4). CMT remains the only reliable screening test to detect infected quarter. It can be easily used at cow-side. A commercially available CMT test reagent kit from "WEIZUR" manufactured by Weizur ® India Private Limited, ANAND, Gujarat is very effective to detect subclinical mastitis in buffaloes. Normally, CMT reaction increases the probability of recovered pathogenic bacteria. Due to lack of cell counting laboratories in field conditions, CMT offers an easy, quick on-site test to indicate the presence of udder infection. Through CMT, not only individual quarter milk samples but also composite milk samples and bulk milk samples can also be tested.

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