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Screening of subclinical mastitis using commercially available WEIZUR test in buffaloes

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Abstract

Subclinical mastitis is a major challenge for milk producing countries like India. A number of infectious agents have been identified but the most common ones are *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli* and *Mycoplasma* species. The infectious nature of mastitis causing agents needs quick and early screening of milk samples. California mastitis test (CMT) is an indirect, on- farm test which is continuously being used by many dairy farms. Subclinical cases of mastitis are hard to detect due to lack of clear-cut systemic sign in udder. The only noticeable sign is fall in milk production. Milk samples from 44 lactating buffaloes were collected and subjected to commercially available WEIZUR test for screening. Results revealed that 83 (69.16%) quarter samples were negative for CMT and 37 (30.83%) quarter samples were positive for CMT. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (++) as 22 (47.82%) and the lowest in CMT score (+++) as 09 (19.56%).

Keywords: Mastitis, buffalo, WEIZUR, milk

Introduction

Milk is the primary source of nutrition for humans. Milk should be fresh and fit for human consumption. Owing to this, screening of milk plays an important role in controlling mastitis. It can be done by a number of tests. One such test which is using by various dairy farms is an on-site test, known as California mastitis test (CMT). In modern times, CMT is considered as a milk grading test in several countries. CMT is one of the oldest test that can detect various forms of udder inflammation under raw conditions. Schalm and Noorlander (1957)^[9] claimed that CMT is a cost effective, easy to use 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 CMT depends upon the number of milk leucocytes as a measure of udder infection (Schalm and Noorlander, 1957)^[9]. The CMT reaction arises by deoxyribonucleic acid originating in cell's nuclei constituting the inflammatory exudates (Carroll and Schalm, 1962)^[1]. The analysis of CMT test is done by the 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). The CMT score of 1 depicts less than one million per milliliter milk leucocytes whereas, a CMT score of 2.2 depict more than one million per millimeter milk leucocytes (Smith and Schultze, 1965)^[10]. The CMT score of 1 to 3 depicts higher cell count count (Leidl *et al.*, 1963)^[3]. Rust *et al.* (2021)^[7] used domestic detergents as CMT reagent and compared the results with the commercially available CMT reagent. Their study depicted that domestic detergents are as good as commercially available CMT reagents and can aid in cost effective detection of mastitis.

Generally, a sodium or potassium salt of long chain fatty acids acting as anionic-surface active agents i.e. alkyl sulphates, alkyl sulphonates, alkyl arylsulphates or alkyl arylsulphonates is the test reagent in CMT (Leach *et al.*, 2008)^[2]. Milk sample and test reagent are poured in equal proportions in CMT paddle with gentle agitation resulting in lysis of somatic cells releasing cellular DNA. The agglutination of DNA gives the sample a 'slimy' or 'mucoïd' texture owing to the number of cells in the sample. The gradation is based on the visible agglutination on an ordinary scale. CMT acts as a qualitative test to measure milk SCC (Moroni *et al.*, 2018)^[5].

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.

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 proper cleaning of udder. The teat ends were swabbed with a cotton wool soaked in 70% ethanol. In the present investigation, 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 comes with a bottle of CMT test Reagent (500 ml) with CMT test paddle and a Dispensing Pump (Figure 1 and 2).

In the four shallow cups of CMT paddle, milk from each teat of buffalo was poured and the paddle was slightly tilted to equalize milk quantities in the cup. The same quantity of CMT reagent was taken into each cup. The paddle was rotated clockwise to mix the sample. The color change and gel formation was recorded. The CMT reagent used in Weizur test is red in color. The purplish or dark purplish color reflects infected milk.



Fig 1-2: WEIZUR® kit showing results from affected milk samples.

Table 1: Observation and interpretation of CMT score

Gel Formation	Color	Score	Result
No gel formation	Grey	0	Normal Milk
Slight gel formation which disappears after rotating the paddle for 10 seconds.	Light Purple	1	Weak Positive (+)
Distinct gel formation which disappears after rotating the paddle for 20 seconds.	Purple	2	Distinct Positive (++)
Gel formation with elevated surface of the mixture	Dark Purple	3	Strong Positive (+++)

Table 2: CMT based Scoring of examined quarters milk samples

Examined Quarters (Sample No.)	Positive Samples		Negative Samples		Score of Positive Quarter Samples					
					+(Weak Positive) (1)		++(Distinct Positive) (2)		+++ (Strong Positive) (3)	
	No.	%	No.	%	No.	%	No.	%	No.	%
120	37	30.83	83	69.16	15	32.60	22	47.82	09	19.56

Table 4: Results of examined animals on CMT

Parameters	Examined Animals (No.)	Percentage (%)
CMT positive	17	38.64
CMT negative	27	61.36
Total	44	100

Results and Discussion

Out of 120 quarter milk samples collected from 44 lactating buffaloes, 83 (69.16%) quarter samples were negative for CMT and 37 (30.83%) quarter samples were positive for CMT (Table 2). With a total of 17 (38.64%) dairy buffaloes were positive for CMT and total of 27 (61.36%) dairy buffaloes were negative for CMT. Among the positive quarter milk samples, the highest incidence was recorded in CMT score (++) as 22 (47.82%) and the lowest in CMT score (+++) as 09 (19.56%). The CMT score reflects the number of leukocytes in milk (Prouty, 1934)^[6]. The positive reaction of CMT depicts alkalinity owing to the increment of milk somatic cells. Similarly, Salama and Saad (2016)^[8] collected 326 milk samples from 90 lactating buffaloes and found that 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%).

Conclusion

From the present investigation, 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.

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