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Comparative studies for efficacy of different diagnostic tests of sub-clinical mastitis in cows

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Abstract
The present study determine the comparative efficacy of different diagnostic tests, 30 cows with 115 quarters were subjected to CMT, WST, SFMT and SCC taking cultural examination as standard, and animal wise incidence was recorded as 86.66%, 73.33%, 70%, 80% and 83.33%, respectively. The quarter wise incidence was 48.69%, 38.26%, 36.52%, 46.08% and 51.30%, respectively. The accuracy of CMT, WST, SFMT and SCC was 76.52, 71.30, 69.56 and 73.39 per cent, respectively, with false positive reactions more with SCC (22.64%) followed by CMT (21.43%), SFMT (21.40%) and least with WST (20.45%). Whereas, false negative reactions were more with SFMT (35.61%) followed by WST (33.80%), SCC (29.03%) and least with CMT (25.42%).

Keywords: comparative efficacy, sub clinical mastitis, cows

Introduction
Subclinical mastitis is bereft of any obvious manifestation of inflammation and is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases and there is change in milk composition [5]. Subclinical mastitis is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds [1]. Besides causing huge losses to milk production, the sub clinically affected animals remain a continuous source of infection to other herd mates [7]. The subclinical form of mastitis in dairy cow s is important because it is 15 to 40 times more prevalent than the clinical form and is difficult to detect, reduces milk production and adversely affects milk quality [14].
The various diagnostic tests for detection of subclinical mastitis are CMT, WST, and SFMT, electrical conductivity of milk, Cl^- estimation in milk, Modified Aulendorfer Mastitis Probe (MAMP) test, somatic cell count and culture [19]. The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell counts (SCC) and microbiological status of the quarter. Though bacteriological culture of milk samples is the standard method for identifying mastitis, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted [16].
California Mastitis Test (CMT) is a simple, inexpensive, rapid and highly sensitive test that accurately predicts the inflammatory cell counts in milk from individual quarters or pooled milk samples [9]. The SCC is a count used to screen epithelial cells that have been shed from the lining of the gland and white blood cells (leucocytes) that have entered the mammary glands in response to injury or infection [2]. SCC is a useful predictor of subclinical udder infection, therefore, it is considered as an important component for assessing the quality and milk hygiene for mastitis control protocols [17]. The leucocyte count is the basis for most indirect tests employed for diagnosis.

Material and Methods
The study was carried out on cases of subclinical mastitis from three different dairy farms and individual holdings during the period from February 2015 to August 2015. All the crossbred cows which have calved more than 15 days were taken for the investigation.
The milk samples from different quarters were subjected to California mastitis test (CMT), White side test (WST), Surf field mastitis test (SFMT) and further subjected to bacteriological examination for isolation of etiological agents.

Comparative efficacy of diagnostic tests
For comparing the efficacy of various diagnostic tests viz., examination of milk by CMT,

WST, SCC and cultural isolation by taking cultural examination as the standard, a group of 30 lactating cows with 115 quarters (5 blind quarters) were randomly selected and subjected to all the above diagnostic tests.

California Mastitis Test (CMT)

The milk samples were subjected to CMT as per the procedure given by Schalm and Noorlander [12] using a modified reagent [15]. The principle of this test is that the detergent causes rupture of somatic cells when added to a milk sample due to which DNA and other cell contents are released. Released DNA from ruptured cells unite to form a gel, the consistency of which depends upon the number of somatic cells.

Procedure

About 2.5 to 3.0 ml of milk was drawn into a plastic paddle with four cups (designed by Glaxo-India) from the quarter. To this, equal volume of the CMT reagent was added. The contents were then mixed by gentle stirring for 15 to 20 sec. Based on the reaction, the results were graded as per Schalm *et al.* [13] as negative, 1+ (there is precipitate but no gel formation), 2+ (the precipitate thickens and forms gel towards the centre of the paddle) and 3+ (distinct gel that adheres to the bottom of the paddle).

White Side Test (WST)

The white side test was performed on the milk samples as per the procedure of Chauhan [4]. The principle of this test is that sodium hydroxide dissolves cell wall of the somatic cells, causing the release of DNA which unite to form viscid masses that break down to form flakes and shreds.

Procedure

In this test, 4-5 drops of the test milk sample was placed on a clean dry glass slide. To this a drop of 4 per cent sodium hydroxide was added and mixed with a glass rod. Milk thickened and flakes appeared when the sample was positive for mastitis. Based on the degree of thickening and appearance of flakes, the results were graded as negative, 1+ (background is less opaque, with larger particles of coagulated materials thickly scattered and slight degree of clumping), 2+ (background is more watery with large clumps of coagulated materials. If the stirring is been rapid, fine threads or strings may be present) and 3+ (background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds).

Surf Field Mastitis Test (SFMT)

This test was performed and scored following the method described by Muhammad *et al.* [10]. This test works on the principle that the DNA of the somatic cells reacts with the detergent (surf) and leads to formation of gel of varying degrees depending on the number of somatic cells in the milk.

Procedure

About 2 ml milk was drawn from bottle into test cup and an estimated 2 ml reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored as negative, 1+, 2+ and 3+ basing on the formation of floccules or gel of varying degrees as per Muhammad *et al.* [10]

Somatic Cell Count (SCC)

Total somatic cell count was estimated using *Ekomilk scan* somatic cells analyser supplied by M/s Eon Traders, as per the manufacturer's instructions. The values obtained were expressed as $\times 10^5$ cells/ml of milk. The milk samples containing SCC range of 0-2 lakh cells/ml were considered negative, 2-4 lakh cells/ml as healthy or sub clinically infected and more than 4 lakh cells/ml as positive for clinical or subclinical mastitis.

Culture

The milk samples from the affected quarters were collected by aseptic precautions into sterile vials and then subjected to bacteriological examination for isolation of etiological agents. Primary identification of bacteria was done based on colony morphology, type of hemolysis and Gram's staining and pure cultures were identified up to genus level as per the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). Gram staining, Motility test, Catalase activity, coagulase test, sugar fermentation test, Haemolysin test, Indole test, Methyl Red test, Vogues– Proskauer test, Citrate utilisation tests were done on a 24-48 hour old pure culture for the identification of bacteria.

Results

To compare the diagnostic efficacy of CMT, WST, SFMT and SCC taking cultural examination as standard, 30 cows with 115 quarters (5 blind) were randomly selected and subjected to the above tests.

The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in depicted in the table 1. According to CMT, WST, SFMT, SCC and cultural examination, the animal wise incidence was 86.66%, 73.33%, 70%, 80% and 83.33%, respectively, whereas quarter wise incidence was 48.69%, 38.26%, 36.52%, 46.08% and 51.30%, respectively

Table 1: Comparative study on the incidence of subclinical mastitis in crossbred cows using different tests.

S. No	Name of Test	Number of animals			Number of quarter		
		Tested	Positive	Incidence (%)	Tested	Positive	Incidence (%)
1.	CMT	30	26	86.66	115	56	48.69
2.	WST	30	22	73.33	115	44	38.26
3.	SFMT	30	21	70	115	42	36.52
4.	SCC	30	24	80	115	53	46.08
5.	Cultural isolation	30	25	83.33	115	59	51.30

California Mastitis Test (CMT)

Various grades of CMT reaction obtained on screening of 115 quarter milk samples by CMT and the infectious status are presented in the table 2. While 59 quarter milk samples were negative for CMT reaction, 15 (25.42%) were culturally positive, further 32, 20 and 4 quarters were showing 1+, 2+

and 3+ CMT reactions out of which 24/32 (75%), 16/20 (80%) and 4/4 (100%) were culturally positive.

Table 2: Grades of CMT reaction VS status of infection of affected quarters (n=115)

S. No	CMT reaction grade	Number of quarters showing CMT reaction	No. of quarters			
			Culturally positive	Per cent	Culturally negative	Per cent
1.	-	59	15	25.42	44	74.58
2.	+	32	24	75	8	25
3.	+ +	20	16	80	4	20
4.	+ + +	4	4	100	0	0
5.	Total	115	59	51.30	56	48.70

White Side Test (WST)

Various grades of WST reaction of 115 quarter milk samples and the infectious status are presented in the table 3. While 71 quarter milk samples were negative for WST reaction, 24 (33.80%) were culturally positive, further 17, 23 and 4 quarters were showing 1+, 2+ and 3+ WST reactions out of which 12/17 (70.59%), 19/23 (82.61%) and 4/4 (100%) were culturally positive.

Table 3: Grades of WST reaction VS status of infection of affected quarters (n=115)

S. No	WST reaction grade	Number of quarters showing WST reaction	No. of quarters			
			Culturally positive	Per cent	Culturally negative	Per cent
1.	-	71	24	33.80	47	66.20
2.	+	17	12	70.59	5	29.41
3.	+ +	23	19	82.61	4	17.39
4.	+ + +	4	4	100	0	0
5.	Total	115	59	51.30	56	48.70

Surf Field Mastitis Test (SFMT)

Different grades of SFMT reaction obtained on screening of 115 quarter milk samples by SFMT and the infectious status are presented in the table 4. While 73 quarter milk samples were negative for SFMT reaction, 26 (35.62%) were culturally positive, further 18, 20 and 4 quarters were showing 1+, 2+ and 3+ SFMT reactions out of which 15/18 (83.33%), 14/20 (70%) and 4/4 (100%) were culturally positive.

Table 4: Grades of SFMT reaction VS status of infection of affected quarters (n=115)

S. No	SFMT reaction grade	Number of quarters showing SFMT reaction	No. of quarters			
			Culturally positive	Per cent	Culturally negative	Per cent
1.	-	73	26	35.62	47	64.38
2.	+	18	15	83.33	3	16.67
3.	+ +	20	14	70	6	30
4.	+ + +	4	4	100	0	0
5.	Total	115	59	51.30	56	48.70

Sensitivity, Specificity and Accuracy

The per cent accuracy of various diagnostic tests for the detection of SCM, with cultural examination as standard are presented in the table 5. The per cent accuracy of CMT, WST, SFMT and SCC was 76.52, 71.30, 69.56 and 73.39 per cent, respectively. The false positive reactions were more with SCC (22.64%) followed by CMT (21.43%), SFMT (21.40%) and least with WST (20.45%), whereas, false negative reactions were more with SFMT (35.61%) followed by WST (33.80%), SCC (29.03%) and least with CMT (25.42%).

Table 6, depicts the sensitivity and specificity of various diagnostic tests. The decreasing order of sensitivity was for CMT (74.58%), SCC (69.49%), WST (59.32%) and SFMT (55.93%) whereas the specificities were highest and same for WST and SFMT (83.93%), followed by SCC and CMT (78.57%), respectively.

Table 5: Per cent accuracy of various diagnostic tests taking cultural examination as standard

Sl. No	Name of the test	Total samples examined	Test positive samples	Test reaction as compared to cultural examination								
				True positive	Per cent	False positive	Per cent	True negative	Per cent	False negative	Per cent	Per cent accuracy
1.	CMT	115	56	44	78.57	12	21.43	44	74.58	15	25.42	76.52%
2.	WST	115	44	35	79.55	9	20.45	47	66.20	24	33.80	71.30%
3	SFMT	115	42	33	78.60	9	21.40	47	64.39	26	35.61	69.56%
4	SCC	115	53	41	77.36	12	22.64	44	70.97	18	29.03	73.39%
5	Cultural isolation	115	59	59	100	-	-	56	100	-	-	100%

$$\% \text{ Accuracy} = \frac{\text{Number of true positive animals} + \text{Number of true negative animals}}{\text{Number of samples examined}} \times 100$$

$$\% \text{ False positive} = \frac{\text{Number of false positive samples}}{\text{Number of samples positive by test}} \times 100$$

$$\% \text{ False negative} = \frac{\text{Number of false negative samples}}{\text{Number of samples negative by test}} \times 100$$

Table 6: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural examination as standard

S. No	Name of the test	Sensitivity	Specificity	Predictive value of positive test
1.	CMT	74.58%	78.57%	78.57%
2.	WST	59.32%	83.93%	79.55%
3.	SFMT	55.93%	83.93%	78.57%
4.	SCC	69.49%	78.57%	77.36%

D+ (Disease present)	D- (Disease negative)
a	b
c	d
a+c	b+d

Positive (T+)
Negative (T-)
a = Disease positive and test positive (true positive)
b = Disease negative but test positive (false positive)
c = Disease positive but test negative (false negative)
d = Disease negative and test negative (true negative)
Sensitivity = a/ (a+c) × 100
Specificity = d/ (b+d) × 100
Predictive value for +ve test = a / (a+b) × 10

Discussion

The per cent accuracy of CMT, WST, SFMT and SCC was 76.52, 71.30, 69.56 and 73.39 per cent, respectively. The per cent accuracy of various diagnostic tests is usually analysed taking into consideration the cultural isolation of mammary pathogens as a standard procedure in the diagnosis of subclinical mastitis. These findings are in agreement with Hoque *et al.* [6] who reported accuracy of 70%, 64.8%, 59.9% and 85.2% for CMT, WST, SFMT and SCC, respectively and concluded that CMT was more reliable than the other two field side tests after the laboratory diagnostic test like SCC. In the present study, false positive reactions were more with SCC (22.64%) followed by CMT (21.43%), SFMT (21.40%) and least with WST (20.45%) and the false negative reactions were more with SFMT (35.61%) followed by WST (33.80%), followed by SCC (29.03%) and least with CMT (25.42%). Langer *et al.* (2014) reported 8.2% and 20.3% false positive reactions and 27.2% and 18.0% false negative reactions with SCC and CMT, respectively, taking culture as standard. Studies of Sharma *et al.* [16] showed 23.79% and 1.63%, false positive and 25.72% and 15.48% false negative reactions with CMT and SCC, respectively, and accuracy of 75.52% for CMT and 91.94% for SCC. Reddy [11] reported 73.33% and 71% accuracy for CMT and SCC, respectively, which are in agreement with the present findings. Siji and Vijayakumar [18] reported that the accuracy of CMT was 83.5%. The results indicate that CMT is a best field side diagnostic indicator taking cultural examination as a gold standard laboratory test.

Conclusion

From this study the sensitivity, specificity and predictive value of different diagnostic tests were studied and it was found that CMT was most sensitive (74.58%) and specificity (83.93%) was highest for WST and SFMT.

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