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SUBCLINICAL MASTITIS IN LACTATING COWS: COMPARISON OF FOUR SCREENING TESTS AND EFFECT OF ANIMAL FACTORS ON ITS OCCURRENCE

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ABSTRACT

A total of 444 quarter milk samples from 111 crossbred dairy cows were subjected to California mastitis test (CMT), somatic cell count (SCC) test, white side test (WST) and surf field mastitis (SFMT) test to quantify their efficacy in detecting sub clinical mastitis in dairy cows of Bangladesh during the period from 2010 to 2011.Milk samples positive by CMT, SCC, WST and SFMT were 265,230, 225 and 222 respectively. All samples were subjected to cultural isolation (gold standard test for comparison of indirect mastitis tests). Of these, 261 samples were positive by cultural isolation. The sensitivity of the CMT, SCC, WST and SFMT were 80.08%, 86.60%, 60.54% and 57.47%; specificity 69.40%, 97.81%, 63.38% and 60.66%; percentage accuracy 75.68%,91.22%, 61.71% and 58.78%; positive predictive value 78.87%, 98.26%, 70.22% and 67.57%; and negative predictive value 70.95%, 83.64%, 52.97% and 50%respectively. Quarter-wise sub clinical mastitis (SCM) was detected in 59.68%, 51.80%, 50.68% and 50% samples by CMT, SCC, WST and SFMT, respectively, while animal-wise SCM was recorded in 72.07%, 66.67%, 64.86% and 61.26% samples by CMT, SCC, WST and SFMT, respectively. The right hind quarters were most significantly ($p<0.001$) susceptible to SCM than other quarters. Cows with 3rd and 4th parity and at their early lactation stage had significantly higher ($p<0.001$) SCM than others. Kappa value of SCC was higher than that of CMT. CMT was concluded to be the most accurate test after cultural isolation and SCC. Unlike laboratory tests as cultural isolation and SCC that require adequate laboratory facilities and skilled personnel, CMT is a reliable diagnostic method in field conditions.

Keywords: Sub clinical mastitis, risk factors, bacteriological culture, California mastitis test, somatic cell count

INTRODUCTION

Bovine mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labour, milk withheld following treatment, death and premature culling (Miller *et al.*, 1993). It is now a well known fact that the sub clinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry in Bangladesh. The annual economic losses occur due to reduced milk production alone caused by SCM in Bangladesh have been estimated to be Taka 122.6 (US $ 2.11) million (Kader *et al.*, 2003). Early detection of mastitis cow is important for most dairy farmers to reduce production losses and to enhance prospects of recovery. The diagnosis of subclinical mastitis is more problematic than clinical mastitis since the milk appears normal but usually has an elevated somatic cell count. Various methods, based on physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of sub clinical mastitis (Batra and Mcallister, 1984). 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. 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. Bacteriological culture of milk identifies the presence of mastitis pathogens but does not provide a measure of the degree of inflammation associated with the infection.

Among the simplest tests, which do not require any complex laboratory equipment, are solutions containing detergents (Sargeant *et al.*, 2001). The California mastitis test (CMT), white side test (WST) and surf field mastitis test (SFMT) are arguably the only reliable cow side screening tests for sub clinical mastitis that can easily be applied. The CMT is a simple, inexpensive, rapid screening test for sub clinical mastitis, based upon the amount of cellular nuclear protein present in the milk sample. Modern mastitis tests allow for indirect determination of the number of somatic cells in milk (Greiner *et al.*, 2000). As the inflammatory process

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develops in udder tissue, the number of these cells (particularly leukocytes) in milk sharply increases. SCC, CMT and intra-mammary infection are associated significantly; therefore these parameters provide the necessary information to evaluate udder health status in cows.

However, the prevalence and different potential risk factors of bovine mastitis are reported by many researchers in Bangladesh (Rahman *et al*., 1997, 2009, 2010, 2014; Rabbani and Samad, 2010; Islam *et al*., 2011; Sarker *et al*., 2013; Barua *et al*., 2014) but there is a lack of reports on comparison of the indirect screening tests in detection of bovine subclinical mastitis along with the risk factors in Bangladesh. The objectives of the present study were to compare the four screening tests for the diagnosis of subclinical mastitis and to find out the relationship between animal risk factors and the occurrence of SCM in lactating cows.

MATERIALS AND METHODS

Animals

The present study was carried out on Rajshahi Dairy and Breed Development Farm, Rajabari, Rajshahi, Bangladesh during the period from 2010 to 2011. A total of 111 cross-bred dairy cows (Sahiwal × Deshi) of different age groups (3 to 11 years), parities (1 to 5) and stage of lactation (early, mid and late) were included in the study group. All cows were housed in tie stall barns and milked twice daily by hand. The regular practices of washing of udder before milking with potassium permanganate solution were used by milkers.

Sample collection

Milk samples (n=444) were collected after proper disinfection of teat surface with 70% ethanol. Sixty ml of milk from all four quarters were collected aseptically in separate sterile polyethylene screw-capped, wide mouth vials after squirting few streams (Buswell, 1995). CMT was conducted at the spot before milk sample collection. The milk samples were placed in an ice box and carried to the laboratory of Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, where they were kept at 4 to 10°C in a refrigerator for further laboratory investigation.

Diagnostic tests

The milk samples were subjected to following diagnostic tests: California mastitis test (CMT), somatic cell count (SCC) test, white side test (WST) and surf field mastitis (SFMT) test. The procedure of CMT was followed in this study as per manufacturer's instruction on quarter fore-milk samples. In brief, 2 ml of milk sample was taken in the CMT paddle and equal quantity of CMT Kit (Leucocytest®, Synbiotics Corporation-2, Lyon, France and marketed by Advance Chemical Co. Bangladesh Ltd.) was added in each cup, rotated for few seconds and the result was recorded within 30 s as 0 (negative), T (trace), 1+, 2++, or 3+++ (Ikram, 1997).

SFMT was performed and scored following the method described by Muhammad *et al.* (1995) in brief, about 2 ml milk was drawn from bottle into test cup and an estimated 2 ml reagent (Surf excel 3% solution in distilled water) 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 1+, 2+ and 3+.

The WST was performed as per procedure described by Kahir *et al.* (2008), in brief, after thorough mixing avoiding violent shaking, 50 µl (five drops) of milk were placed on a glass slide with a dark background by micropipette. Subsequently 20 µl of WST reagent (4% NaOH) were added to the milk sample and the mixture was stirred rapidly with a toothpick for 20-25 seconds. A breaking up of milk in flakes, shreds and viscid mass was indicative of positive reaction. On the other hand, milky and opaque and entirely free of precipitant was indicative of negative reaction. The SCC test was performed according to Schalm *et al.* (1971).

The milk sample was mixed thoroughly and the cream was dispersed throughout the specimens. A measured drop (0.01ml/10µl) by means of micro-pipette was then spread evenly over an area of one square centimeter on a microscopic glass slide. The milk film was allowed to air dry. Then the fat was removed from dried film in the following way: The slides were dipped in Xylene for 1 to 2 minutes. Later they were dried and immersed in 95% ethyl alcohol for 2 to 5 minutes and drain dry. Then immerse in Broadhurst-Paley stain for 5 seconds, or longer if necessary, to obtain proper intensity of staining. Then briefly rinse the slide in water and drain dry. Ten microscopic fields were counted by using oil immersion objective (10×100mm) for the determination of the number of the leukocyte cells present. The required number of fields is counted by moving the slide horizontally from one edge of the film through the center to the opposite edge. The second count is made vertically through the film in the same manner. The total number of the leukocyte cells counted is multiplied by the Working Factor (WF) of the particular microscope to obtain the no. of cells/ml of milk.

According to Schalm *et al.* (1971), the relationship of Working Factor (WF) with the number of fields to be counted using an oil immersion objective having a field diameter of 0.016 cm given below:

Total No. in 1 field × 500,000 = No. per ml
Total No. in 10 field × 50000 = No. per ml
Total No. in 50 field × 10,000 = No. per ml
Total No. in 100 field × 5,000 = No. per ml

The following criteria are used in making the cell count:

1. Count all nucleated somatic cells within a field including those at the periphery with more than 50% of the cell body in view.
2. Free nuclei representing more than 50% of the nuclear material are counted.
3. A cytoplasmic mass without nucleus and small cell fragments with little nuclear material are not counted.

Animals were considered positive for mastitis when CMT, WST and SFMT score was ≥1+ and SCC value was $\geq 2\times10^5$/ml of milk (threshold value). The samples (n=444) were also subjected to bacteriological culture as per Quinn *et al.* (1994).

The following diagnostic test characteristics were determined using the milk bacteriological culture result as a gold standard control.

$$Accuracy = \frac{\mathbf{TP + TN}}{\mathbf{TP + FP + FN + TN}} \times 100$$

$$Sensitivity = \frac{\mathbf{TP}}{\mathbf{TP + FN}} \times 100$$

$$Specificity = \frac{\mathbf{TN}}{\mathbf{TN + FP}} \times 100$$

$$Positive\ Predictive\ Value = \frac{\mathbf{TP}}{\mathbf{TP + FP}} \times 100$$

$$Negative\ Predictive\ Value = \frac{\mathbf{TN}}{\mathbf{TN + FN}} \times 100$$

Where: TP =True Positive, FP =False Positive, TN =True Negative, FN =False Negative.

Statistical analysis

The percentage accuracy of the tests and sensitivity, specificity, and the predictive values of the CMT, SCC, WST and SFMT results, compared to culture results, were calculated using standard two-by-two contingency tables. Correlations between the dependent variables were calculated using Pearson's correlation. Data were also analyzed by Chi-square test to observe the significant influence of parity, lactation stage on subclinical mastitis of cows.

RESULTS AND DISCUSSION

Percentage accuracy, agreement and correlation of various indirect tests

Among the 444 quarter milk samples tested for SCM in lactating cows, 265 milk samples were CMT positive and 179 samples were CMT negative. Milk samples positive by SCC, WST and SFMT were 230, 225 and 222 respectively. The accuracy of CMT, SCC, WST and SFMT was 75.68%, 91.22%, 61.71% and 58.78% (Table 1). The sensitivity, specificity, positive and negative predictive values and likelihood ratios are given in Table 2. In the present investigation, the sensitivity and specificity of the SCC were higher than those of CMT, WST and SFMT. The sensitivity and specificity of SFMT were lower than those of other. SCC showed the highest correlation (r=0.831) with bacterial culture test, followed by CMT, WST and SFMT. It is accepted that Kappa values <0.4 indicate poor agreement, values between 0.4 and 0.75: fair to good agreement and values >0.75:

excellent agreement. Thus, SCC exhibited excellent agreement with bacterial culture test while CMT had fair to good agreement (Table 2). In the present study it was apparent that SCC was the most reliable test and closest

Table 1. Percentage accuracy of various indirect tests used for the diagnosis of mastitis

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy
CMT	444	265 (59.68%)	209 (78.87%)	56 (21.13%)	127 (70.95%)	52 (29.05%)	75.68%
SCC	444	230 (52%)	226 (98.26%)	4 (1.74%)	179 (83.64%)	35 (16.36%)	91.22%
WST	444	225 (50.68%)	158 (70.22%)	67 (29.77%)	116 (52.97%)	103 (47.03%)	61.71%
SFMT	444	222 (50%)	150 (67.57%)	72 (32.43%)	111 (50%)	111 (50%)	58.78%

TP=True Positive, FP=False Positive, TN=True Negative, FN=False Negative.

Table 2. Agreement and correlation of various indirect tests used for the diagnosis of mastitis with bacteriological (cultural) examination

Test	Sensitivity %	Specificity %	PPV %	NPV %	PLR	NLR	κ	r %	p
CMT	80.08	69.40	78.87	70.95	2.617	0.287	0.496	49.6	0.00
SCC	86.60	97.81	98.26	83.64	39.543	0.137	0.823	83.1	0.00
WST	60.54	63.38	70.22	52.97	1.653	0.623	0.232	23.6	0.00
SFMT	57.47	60.66	67.57	50	1.461	0.701	0.176	17.8	0.00

PPV=Positive Predictive Value, NPV=Negative Predictive Value, PLR=Positive Likelihood Ratio, NLR=Negative Likelihood Ratio,κ =Kappa (measure of agreement), r=Pearson's Correlation.

to the bacteriological results. Present findings are in agreement with Sharma *et al.* (2008). They reported that SCC was the most accurate test for the diagnosis of subclinical mastitis followed by the modified California mastitis test (MCMT) and the modified White side test (MWST). Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by MWST (79.74%). Reddy *et al.* (1998) compared the specificity and sensitivity of CMT and SCC with standard cultural test and observed 100% predictive value with the cultural test of the milk, 84.84% specificity for SCC and 73.30% for CMT. Tanwar *et al.* (2001) also compared various diagnostic tests for detection of subclinical mastitis and indicated 100% sensitivity for SCC and 96% for CMT reaction. According to Goswami *et al.* (2003), animal-wise efficacy of indirect tests taking cultural examination as a standard was in the following descending order: SCC (97.46%), MCMT (69.62%) and MWST (63.29%). So, the present findings regarding accuracy and sensitivity of indirect tests for detection of subclinical mastitis support the earlier observations. From a study of SCC and CMT sensitivity and specificity for identifying intramammary infection in early lactation Sargeant *et al.* (2001) concluded that CMT could be used in dairy herd monitoring program as a screening test to detect fresh cows with intramammary infection caused by major pathogens. Barbosa *et al.* (2002) reported that the SCC and CMT are dependent and were highly correlated for diagnosis of subclinical mastitis. Sudhan *et al*. (2005) reported that the percentage of agreement of CMT, SLST, bromothymol blue card test and White side test with bacteriological examination were 57.89%, 62.07%, 64.46% and 68.65% respectively.

Prevalence of subclinical mastitis in lactating cows

In the present study, quarter-wise 59.68%, 51.80%, 50.68% and 50.00% and animal-wise as 72.07%, 66.67%, 64.86% and 61.26% of samples were found positive by CMT, SCC, WST and SFMT respectively (Table 3). The quarters were found positive for SCM of different grades. Quarter side-wise prevalence of SCM was shown in Table 4. The highest prevalence of SCM was found in Right Hind (RH) quarter by all four tests that are 72.07%, 64.86%, 63.06% and 62.16% by CMT, SCC, WST and SFMT. In RH quarter, the prevalence of subclinical mastitis was significantly higher (p<0.001) than other quarter (quarter-wise). Lowest prevalence of SCM was found in Left Front (LF) quarter that represent as 45.05%, 39.64%, 36.04%

Table 3. Prevalence and severity of subclinical mastitis in lactating cows

Sl. No.	Test used	Types	Total No. tested	Positive, No. (%)				Negative No. (%)	Level of significance
				1+	2+	3+	Total		
1.	California Mastitis Test (CMT)	Cows	111	42 (52.25)	27 (33.75)	11 (13.75)	80 (72.07)	31 (27.93)	NS
		Quarters	444	146 (55.09)	82 (30.94)	37 (13.96)	265 (59.68)	179 (40.32)	**
2.	Somatic cell count (SCC)	Cows	111	40 (54.05)	27 (36.487)	7 (9.47)	74 (66.67)	37 (33.33)	NS
		Quarters	444	149 (64.78)	58 (25.22)	23 (10.00)	230 (51.80)	214 (48.20)	**
3.	White Side Test (WST)	Cows	111	39 (54.17)	26 (36.11)	7 (9.72)	72 (64.86)	39 (35.14)	NS
		Quarters	444	146 (64.87)	56 (24.87)	23 (10.22)	225 (50.68)	219 (49.32)	**
4.	Surf Field Mastitis Test (SFMT)	Cows	111	37 (54.41)	25 (36.76)	6 (8.82)	68 (61.26)	43 (38.74)	NS
		Quarters	444	144 (64.86)	55 (24.78)	23 (10.36)	222 (50.00)	222 (50.00)	**

**Significant at 1% level (p<0.01)

and 35.14% by CMT, SCC, WST and SFMT respectively. The results are in agreement with Sudhan *et al.* (2005) who reported that the right hind quarter was the most affected (38.18%) compared with the other quarters. Lee-and Lee (2007) reported that the mean Somatic Cell Score (SCS) of milk samples from the front quarters was lower than those of the milk samples from the rear quarters. Sharma *et al.* (2007) who reported single and hind quarter involvement was maximum in case of subclinical mastitis.

Prevalence of subclinical mastitis on the basis of parity

On the basis of parity the prevalence of subclinical mastitis is presented in Table 5. An increased prevalence of SCM with increased number of parity was observed in this study. The highest prevalence of SCM was found at 3^{rd}-4^{th} parity by all four tests that are 71.43%, 64.16%, 64.29% and 63.39% by CMT, SCC, WST and SFMT respectively (quarter-wise) and 82.14%, 75.00%, 71.43% and 71.43% by CMT, SCC, WST and SFMT respectively (animal-wise). In 3^{rd} - 4^{th} parity, the prevalence of subclinical mastitis was significantly higher (p<0.001) than other no. of parity (quarter wise). The lowest prevalence of SCM was found at 1^{st} parity by all four tests that are 21.88%, 15.62%, 21.88% and 20.31% by CMT, SCC, WST and SFMT respectively (quarter-wise) and 43.75%, 43.75%, 50% and 43.75% by CMT, SCC, WST and SFMT respectively (animal-wise).

Table 4. Quarter-wise prevalence of subclinical mastitis in lactating cows

Test used	Quarter side	No. of samples	Negative No. (%)	Positive, No. (%)				Level of significance
				1+	2+	3+	Total	
California Mastitis Test (CMT)	LF	111	61(54.95)	30(27.03)	13(11.71)	7(6.31)	50(45.05)	
	LH	111	36(32.43)	39(35.14)	25(22.5)	11(9.91)	75(67.57)	
	RF	111	51(45.95)	39(35.13)	14(12.61)	7(6.31)	60(54.05)	
	RH	111	31(27.93)	38(34.23)	30(27.03)	12(10.1)	80(72.07)	**
	Total	444	179(40.39)	146(32.88)	82(18.47)	37(8.33)	265(59.68)	
Somatic cell count (SCC)	LF	111	67(60.36)	27(24.32)	13(11.7)	4(3.6)	44(39.64)	
	LH	111	43(38.74)	46(41.44)	16(14.1)	6(5.41)	68(61.26)	
	RF	111	65(58.56)	29(26.13)	12(10.81)	5(4.5)	46(41.44)	**
	RH	111	39(35.14)	47(42.34)	17(15.32)	8(7.2)	72(64.86)	
	Total	444	214(48.2)	149(33.55)	58(13.07)	23(5.18)	230(51.8)	
White Side Test (WST)	LF	111	71(63.96)	25(22.52)	11(9.91)	4(3.61)	40(36.04)	
	LH	111	46(41.44)	42(37.84)	16(14.41)	7(6.31)	65(58.56)	
	RF	111	61(54.95)	34(30.64)	12(10.81)	4(3.6)	50(45.05)	
	RH	111	41(36.94)	45(40.54)	17(15.32)	8(7.2)	70(63.06)	**
	Total	444	219(49.32)	146(32.88)	56(12.62)	23(5.18)	225(50.68)	
Surf Field Mastitis Test (SFMT)	LF	111	72(64.86)	23(20.72)	12(10.82)	4(3.6)	39(35.14)	
	LH	111	47(42.34)	44(39.64	14(12.61)	6(5.41)	6(57.66)	
	RF	111	61(54.95)	33(29.73)	12(10.81)	5(4.5)	50(45.66)	
	RH	111	42(37.84)	44(39.64)	17(15.32)	8(7.2)	69(62.16)	**
	Total	444	222(50.00	144(32.43)	55(12.39)	23(5.18)	222(50.00)	

**Significant at 1% level ($p<0.01$)

LF=Left front quarter, LH=Left hind quarter, RF =Right front quarter, RH=Right hind quarter

This observation supports with the reports of Rasool *et al.* (1985), Devi *et al.* (1997) who reported an increasing prevalence of SCM with advancing parity. Batra and Mcallister (1984) reported that Somatic Cel1 Count and CMT score increased from first to fourth parity while there was general trend for increasing conductivity from second to fourth parity. Lee and Lee (2007) reported that increased SCC was observed with increase in parity.

Prevalence of subclinical mastitis on the basis of lactation stage

The prevalence of subclinical mastitis on the basis of lactation stage is shown in Table 6. It appears from the Table 6 that, all three stages of lactating cows were affected with subclinical mastitis. The highest prevalence of SCM was found at early lactation of stage by all four tests that are 78.43%, 76.47%, 70.59% and 68.63% by CMT, SCC, WST and SFMT respectively (animal-wise) and 70.58%, 64.22%, 63.24% and 61.76% by CMT, SCC, WST and SFMT respectively (quarter-wise). In early stage of lactation, the prevalence of subclinical mastitis was significantly higher ($p<0.001$) than other stage of lactation (quarter-wise).

The lowest prevalence of SCM was found at mid and late lactation stage by CMT, SCC, WST and SFMT tests that are 65.63%, 56.25%, 59.37% and 53.57% (animal-wise) and 49.22%, 39.06%, 39.06%, and 39.29 % (quarter-wise). This observation supports with the reports of Jyoti *et al.* (1998) who reported 77.77%, 63.76% and 41% prevalence of SCM in early, mid and late lactation respectively in an organized farm. Rahman *et al.* (1997) reported that highest prevalence of SCM during the 3rd months (34.00%) of lactation. Lalrintluanga *et al.* (2003) reported that mastitis incidence was higher during the early stage of the 3rd lactation (36.60%). However, SCC is found out to increase in the first few days of lactation and may be high up to the first month of lactation (Atakan, 2008) and increase towards the end of lactation is considered to be physiological. Sederevicius *et al.* (2006) reported a temporary increase in SCC just after calving due to adaptation of the udder from non-lactating to lactating status, while in mid lactations SCC usually remains in normal range.

Table 5. Effect of parity on the prevalence of subclinical mastitis in lactating cows

Test	Parity	Total animal	Animal-wise Positive No. (%)	Animal-wise Negative No. (%)	Level of significance	Total quarter	Quarter-wise Positive No. (%)	Quarter-wise Negative No. (%)	Level of significance
CMT	1ST	16	7(43.75)	9(56.25)	NS	64	14(21.88)	50(78.12)	**
	2nd	17	10(58.82)	7(41.18)		68	30(44.12)	38(55.88)	
	3rd	30	24(80.00)	6(20.00)		120	85(70.83)	35(29.17)	
	4th	28	23(82.14)	5(17.86)		112	80(71.43)	32(28.57)	
	5th	20	16(80.00)	4(20.00)		80	56(70.00)	24(30.00)	
	Total	111	80(72.07)	31(27.93)		444	265(59.68)	179(40.32)	
SCC	1ST	16	7(43.75)	9(56.25)	NS	64	10(15.62)	54(84.38)	**
	2nd	17	9(52.94)	8(47.06)		68	24(35.29)	44(64.71)	
	3rd	30	22(73.33)	8(26.67)		120	77(64.16)	43(35.84)	
	4th	28	21(75.00)	7(25.00)		112	70(62.50)	42(37.50)	
	5th	20	15(75.00)	5(25.00)		80	49(61.25)	31(38.75)	
	Total	111	74(66.67)	37(33.33)		444	230(51.8)	214(48.20)	
WST	1ST	16	8(50.00)	8(50.00)	NS	64	14(21.88)	54(78.12)	**
	2nd	17	10(58.82)	7(41.18)		68	28(41.18)	40(58.82)	
	3rd	30	20(66.67)	10(33.33)		120	68(56.67)	52(43.33)	
	4th	28	20(71.43)	8(28.57)		112	72(64.29)	40(35.71)	
	5th	20	14(70.00)	6(30.00)		80	43(53.75)	37(46.25)	
	Total	111	72(64.86)	39(35.14)		444	225(50.68)	219(49.32)	
SFMT	1ST	16	7(43.75)	9(56.25)	NS	64	13(20.31)	51(79.69)	**
	2nd	17	9(52.94)	8(47.06)		68	29(42.65)	39(57.35)	
	3rd	30	18(60.00)	12(40.00)		120	65(54.17)	55(45.83)	
	4th	28	20(71.43)	8(28.57)		112	71(63.39)	41(36.61)	
	5th	20	14(70.00)	6(30.00)		80	44(55.00)	36(45.00)	
	Total	111	68(61.26)	43(38.74)		444	222(50.00)	222(50.00)	

**Significant at 1% level ($p<0.01$)

Table 6. Effect of stage of lactation on the prevalence (animal-wise and quarter-wise) of subclinical mastitis in lactating cow

Test	Lactation stage	Total animal	Animal-wise		Level of significance	Total quarter	Quarter-wise		Level of significance
			Positive No. (%)	Negative No. (%)			Positive No. (%)	Negative No. (%)	
CMT	Early (6-90d)	51	40 (78.43)	11 (21.57)	NS	204	144 (70.58)	60 (29.42)	**
	Mid (91-180d)	28	19 (67.86)	9 (32.14)		112	58 (51.79)	54 (48.21)	
	Late (>180d)	32	21 (65.63)	11 (34.37)		128	63 (49.22)	65 (50.78)	
	Total	111	80 (72.07)	31 (27.93)		444	265 (59.68)	179 (40.32)	
SCC	Early (6-90d)	51	39 (76.47)	12 (23.53)	NS	204	131 (64.22)	73 (35.78)	**
	Mid (91-180d)	28	17 (60.71)	11 (39.29)		112	49 (43.75)	63 (56.25)	
	Late (>180d)	32	18 (56.25)	14 (43.75)		128	50 (39.06)	78 (60.93)	
	Total	111	74 (66.67)	37 (33.33)		444	230 (51.80)	214 (48.20)	
WST	Early (6-90d)	51	36 (70.59)	15 (29.41)	NS	204	129 (63.24)	75 (36.76)	**
	Mid (91-180d)	28	17 (60.71)	11 (39.29)		112	46 (41.07)	66 (58.93)	
	Late (>180d)	32	19 (59.37)	13 (40.63)		128	50 (39.06)	78 (60.94)	
	Total	111	72 (64.86)	39 (35.14)		444	225 (50.68)	219 (49.32)	
SFMT	Early (6-90d)	51	35 (68.63)	16 (31.37)	NS	204	126 (61.76)	78 (38.24)	**
	Mid (91-180d)	28	15 (53.57)	13 (46.43)		112	44 (39.29)	68 (60.71)	
	Late (>180d)	32	18 (56.25)	14 (43.75)		128	52 (40.63)	76 (59.37)	
	Total	111	68 (61.26)	43 (38.74)		444	222 (50.00)	222 (50.00)	

**Significant at 1% level ($p<0.01$)

In conclusion, SCC was the most accurate test after cultural isolation, followed by CMT, WST and SFMT. Taking into consideration that laboratory tests such as cultural isolation and SCC require adequate laboratory facilities, personnel and time, CMT is a reliable diagnostic method for use as a regular mastitis screening test in field conditions even by less trained dairymen.

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