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REGULAR ARTICLES

Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh

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Abstract Identification of cows with subclinical mastitis (SCM) is an important tool for sustainable dairying and implementing effective mastitis control strategies. A total of 892 quarters milk samples from 228 lactating cows were screened by California mastitis test (CMT), White side test (WST), Surf field mastitis test (SFMT), and somatic cell count (SCC) to study the prevalence of bovine SCM in some selected areas of Bangladesh. Out of 228 cows, 148 (64.9 %), 138 (60.5 %), 132 (57.9 %), and 164 (71.9 %) were found positive for SCM by CMT, WST, SFMT, and SCC, respectively. The prevalence of bovine SCM was diagnosed 45.7, 40.2, 36.6, and 29.6 % in Chittagong, Sirajgonj, Mymensingh, and Gazipur districts, respectively, based on a combination of all tests. The overall quarter-wise prevalence of SCM was 45.7, 43.5, 41.2, and 55.0 % for CMT, WST, SFMT, and SCC. Single quarters and left front quarters were more prone to SCM ($P<0.05$). Friesian crossbred cows (56.4 %), BCS 2.0–2.5 (55.4 %), and parity 4–6 (52.4 %), the late lactation stage (5–8 months; 64.7 %) and high yielding cows (16–20 L/day; 65.3 %) were more susceptible to SCM ($P<0.05$). The sensitivity of the CMT, WST, SFMT, and SCC was 65.8, 57.9, 51.0, and 82.5 %; specificity 76.2, 72.4, 69.5, and 89.4 %; percentage accuracy 70.0, 64.8, 59.9, and 85.2 %; positive predictive value 75.2, 69.8, 64.9, and 92.7 %, respectively. The categories of CMT reactions were strongly correlated with SCC ($P<0.05$). Kappa value of SCC was higher than that of other tests (SCC>CMT>WST>SFMT). Thus, CMT was concluded to be the most accurate ($r=0.782$) field diagnostic test after laboratory test like SCC ($r=0.924$). However, the use of any single test may not be reliable in diagnosing SCM, while the result of CMT supported by SCC might be used effectively to pinpoint diagnosis of SCM in dairy animals than alone.

Keywords Screening tests · Subclinical mastitis · Somatic cell count · Cows

Introduction

Subclinical mastitis (SCM) has been remaining as one of the first observed dairy cattle diseases alike to clinical mastitis since the inception of cattle domestication over 5000 years ago. Bovine SCM is an important production disease of dairy animals and can directly or indirectly affect the economy of the farmers all over the world including developed countries (Sharma et al. 2012). According to the National Mastitis Council (1996), the total economic losses due to mastitis include value of reduced milk production (70 %), premature culling (14 %), veterinary expenses (9 %), and milk discarded or low graded (7 %).

SCM is one of the most persistent and widely spread disease conditions of dairy cattle with importance to milk hygiene and quality (Ogola et al. 2007). Despite its economic importance, mastitis also carries public health significance, more importantly in relation to drug residue in milk and passage of pathogenic organisms to humans leading to zoonotic diseases (Sharma et al. 2007; Sharif et al. 2009). SCM in dairy cows is important because it (a) is 15 to 40 times more prevalent than the clinical form, (b) usually precedes the clinical form, (c) is of long duration, (d) is difficult to detect,

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(e) reduces milk production, and (f) adversely affects milk quality (Seegers et al. 2003).

The prevalence of SCM can range from 29.34 to 78.54 % in cows (Sharma and Maiti 2010) and 66–70.32 % in buffaloes (Sharma et al. 2007). However, in Bangladesh, the prevalence of bovine SCM was reported from 42.0 to 57.0 % (Kader et al. 2002) and 55.0 % (Jha et al. 2010). SCM is a problem not only in the individual cow level but also in herd level, and thus, the herd needs to be tested periodically for the prevalence of SCM. SCM is by far the more costly disease in the majority of herds and is often defined as the presence of a microorganism in combination with an elevated somatic cell count of the milk.

Bangladesh is endowed with more than 12 million dairy cows (out of 25 million cattle population), the dairy industry of this country has not yet got sustainability and remained underdeveloped. Both clinical and subclinical forms of mastitis have been significantly constrained for the development of the dairy industry herein Bangladesh. Most of the dairy farmers are not sentient of the different easiest and effective screening tests like California mastitis test (CMT), White side test (WST), Surf field mastitis test (SFMT), and milk somatic cell count (SCC) for the diagnosis of SCM. Moreover, these dairy farmers are not always aware of the proper control and management of SCM. SCC is a useful predictor of intramammary infection, and therefore, an important component of milk quality assessment, hygiene, and mastitis control. Very few studies have been undertaken to demonstrate the prevalence of bovine SCM through screening tests (CMT, WST, and SFMT) and SCC in the smallholders of dairy farms in Bangladesh. Furthermore, till today, there is a lack of research information regarding the application SCC to diagnose SCM in zebu and crossbred cows in Bangladesh. On the other hand, many dairy producers of Bangladesh fail to completely understand the implications of SCC for udder health or how high SCC can affect production and quality of milk.

The present study was therefore undertaken to investigate the prevalence of bovine SCM through different screening tests (CMT, WST, and SFMT) and SCC in some selected areas of Bangladesh.

Materials and methods

Selection of dairy farms and data collection

The study was conducted in smallholders of dairy farms in Chittagong, Sirajgonj, Mymensingh, and Gazipur districts of Bangladesh. Data from 228 lactating dairy cows regarding their age, breed, body condition score, parity, lactation stage, per day milk yield, and history of previous clinical or subclinical mastitis were investigated and recorded in a structured questionnaire.

Collection of milk samples

After proper sanitization of teat orifice with 70 % ethyl alcohol, 10–20 mL of milk samples from all four quarters viz. left fore (LF), left hind (LH), right fore (RF), and right hind (RH) were collected aseptically following squirting first few streams in sterile screw-capped plastic tube. A portion of the squirted milk samples were screened by CMT, WST, and SFMT, and rest of the milk samples were then kept in ice box and carried to the laboratory, where the milk samples were kept at 4–8 °C in a refrigerator for further laboratory investigation. After complete milking, clinical examination of udder and teats was performed as described by Jha et al. (2010).

Screening tests

CMT was performed following the modified procedure described by Jha et al. (2010) and Lesile et al. (2002) along with the manufacturer's instruction (CMT®, Original Schalm reagent, ThechniVet, USA). In brief, about 2 mL of milk sample was squirted in each cup of mastitis paddle, and an equal volume (2 mL) of CMT reagent was added to the cups. The reactions were developed within 10 s in positive samples and scoring was carefully done. CMT positive cows (score≥+1) were defined as having at least one positive quarter for SCM.

The WST was performed as per procedure described by Kahir et al. (2008). In brief, 5 mL of milk sample was placed on each cup, and 2 mL of WST reagent (4 % NaOH solution) was added to the cups. A breaking up of milk in flakes, shreds, and viscid mass was indicative of positive reaction.

SFMT was performed and scored following the method described by Muhammad et al. (1995). In brief, 2 mL milk sample was drawn in to each cup and 2 mL reagent (4 % solution of Surf Excel®, Unilever Bangladesh Ltd.) into each cup. The mixture was swirled up to 1 min, and appearance of floccules or gel of varying degrees was interpreted as a positive SFMT reaction for having SCM.

Somatic cell count

The SCC (cells/mL) for the quarter milk samples was determined using NucleoCounter SCC-100 (Coulter electronic–ChemometecA/s, Denmark) following the protocol developed by Saleh and Faye (2011). In brief, mixing of the representative cell sample (milk) was done with equal volume of Reagent A (lysis/disaggregation buffer) and Reagent B (stabilizing buffer). The NucleoCassette was loaded with the lysate solution by immersing the tip of the cassette into the solution and pressing the piston. The NucleoCassette was then placed in the instrument and pressing of the "Run" key. After

30 s, the cell count was presented on the instrument display. Optionally, data were transferred to an external PC using USB connection or printed on an external printer.

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

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

$$\text{Specify} = \frac{\text{TN}}{\text{FP} + \text{TN}} \times 100$$

$$\text{Positive predictive value} = \frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$$

Here,

TP true positive
FP false positive
TN true negative
FN false negative

Data analysis

The data were entered into Microsoft Excel worksheet (office, 2007) and exported to SPSS (version 11.5) for analysis. The descriptive statistics were calculated. The percentage accuracy, sensitivity, specificity, positive predictive value, and Kappa index (% agreement) of CMT, WST, and SFMT results compared to SCC were calculated by using two-by-two contingency table. Univariate analysis in general linear model (GLM) procedure was conducted to evaluate the effects of different subgroups (different variables of each factor) within a single group (factors affecting the prevalence: breeds, age, BCS, parity, lactation stage, milk yield). Duncan's multiple range was done to compare the means and $P<0.05$ was considered significant. Correlations between the dependent variables were calculated using Pearson's correlation.

Results

The results of the present study showed that 148 (64.9 %) cows were positive for SCM by CMT, while 138 (60.5 %), 132 (57.9 %) and 164 (71.9 %) cows were SCM positive by WST, SFMT, and SCC, respectively (Table 3). The prevalence of SCM was recorded highest (45.7 %) in Chittagong district and lowest (29.6 %) in Gazipur district in the combination of SCC and other three screening tests: CMT, WST, and SFMT (Fig. 1). In this study, the overall quarter-wise prevalence of bovine SCM was 45.7, 43.5, 41.2, and 55.0 % for CMT, WST, SFMT, and SCC, respectively (Table 3). Among 892 quarters, 20 (2.2 %) quarters were found blind and could not be subjected to any test for SCM. On an average, the front quarters were more susceptible to SCM than the rear quarters, and LF quarters were mostly affected with SCM in our current investigation (Table 3). Single quarter involvement to SCM was maximum (52.0 %), followed by double, triple, and quadruple quarters.

Crossbred Friesian cows were more (56.4 %) vulnerable for SCM than the other breeds (Table 2) and differences among the breeds of cows were significant ($P<0.05$). This study exposed that the prevalence of bovine SCM varied according to the BCS, parity, stage of lactation, per day milk yield, and age of animals (Table 2). Cows having BCS 2.0–2.5 (55.4 %), 6–8 years of age (50.5 %), parity 4–6 (52.4 %), late lactation stage (5–8 months; 64.7 %), and high milk yield (16–20 L/day; 65.3 %) were more susceptible to SCM ($P<0.05$).

In our current investigation, CMT was found as the best test regarding the episode of diagnostic reaction (Fig. 2), and the categories of CMT were strongly related to SCC (Table 1;

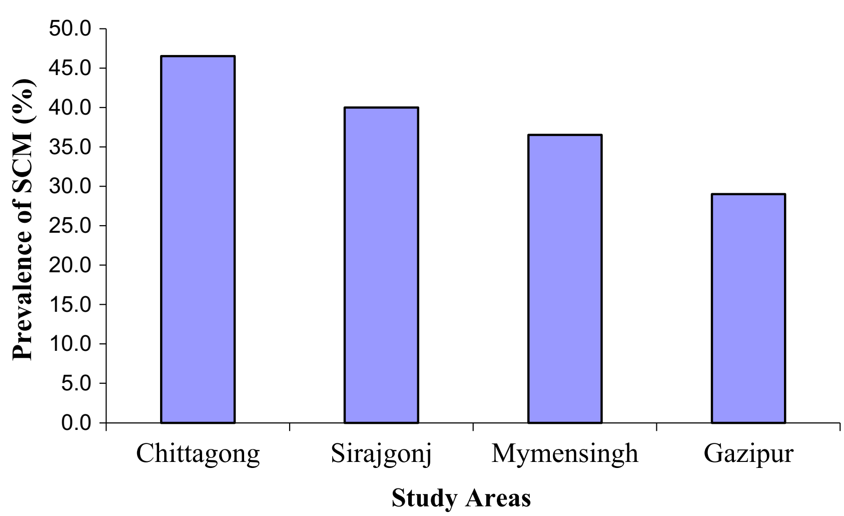

Fig. 1 Prevalence of subclinical bovine mastitis in selected areas of Bangladesh based on a combination of SCC, CMT, WST, and SFMT results

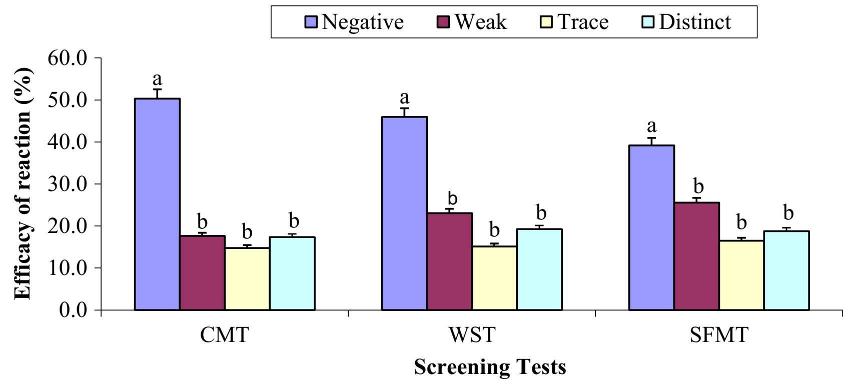

Fig. 2 Efficacy of reactions among three screening tests in milk samples. Values of the different screening tests (*a*, *b*) differ significantly

$P<0.05$). The diagnostic accuracy of CMT, WST, SFMT, and SCC was 70.0, 64.8, 59.9, and 85.2 %, respectively (Table 4). The sensitivity and specificity of the SCC were higher than those of CMT, WST, and SFMT. The lowest specificity (69.5 %) was found for SFMT. Kappa index (% agreement) of all four tests on various combinations of gold standard (SCC) is shown in Table 4. It is accepted that Kappa values <0.4 indicate poor agreement, values between 0.4 and 0.75 indicate fair to good agreement, and values >0.75 indicate excellent agreement. Among the tests, percentage of agreement (Kappa value) was found highest ($k=0.88$) for SCC followed by CMT, WST, and SFMT (SCC>CMT>WST>SFMT; Table 4). CMT showed the highest correlation ($r=0.782$) with SCC ($r=0.924$), followed by WST ($r=0.683$) and SFMT ($r=0.566$). Thus, CMT exhibited excellent agreement with SCC while WST and SFMT had fair to good agreement.

The results of the present study explored that there were marked variations in somatic cell counts according to the breeds, BCS, age, parity, lactation stage, and per day milk yield of the cows ($P<0.05$) (Table 2). On an average, the left front (LF) quarters had the highest milk somatic cell counts than the other quarters, and the mean SCC was 6.7, 8.6, 5.3, and 5.8×10^5 cells/mL of milk samples for RF, LF, RR, and LR quarters, respectively (Fig. 3). On an individual basis, it can be seen that the LF quarters were more affected with SCM compared to RF quarter as reflected by the different tests (Table 3). Of the 892 samples, SCC obtained the highest percentage of accuracy, sensitivity, and specificity compared to the other indirect tests done for SCM diagnosis (Table 4).

Table 1 Milk somatic cell count (Mean±SEM) according to the total CMT scores in four quarters of cows udder

Categories of CMT results	No. of SCs (Mean±SEM)×10^5/mL milk samples
1 (Negative)	1.6a±0.1
2 (Trace)	4.4b±0.2
3 (Weak)	6.0c±0.6
4 (Distinct)	12.9d±0.5
5 (Strong)	17.7e±0.3

Values with the different lowercase Roman letters (a, b, c, d, and e) within the same column differ significantly at least by $P<0.05$

Discussion

The knowledge and effectiveness of different screening tests to detect subclinical form of mastitis have long been acknowledged as an imperative to the success of mastitis control and treatment either in individual cows or in herd level. The efficacy of field diagnostic tests especially CMT to screen cows was found close to that of laboratory techniques like SCC. Out of 228 lactating cows examined in this investigation, 64.9 % cows were found positive for SCM by CMT, while WST, SFMT, and SCC diagnosed SCM 60.5, 57.9, and 71.9 % in cows, respectively. These animal basis findings were closely proximated with the observations of Shahid et al. (2011) who reported 56.8 % occurrence of SCM in dairy cows by WST and SFMT. Our results are also supported by the findings of Sharma et al. (2007; 2010) who recorded 66.0, 68.6, and 72.0 % prevalence of SCM in buffaloes by MWST, MCMT, and SCC, respectively. Goswami et al. (2003) also reported that animal-wise efficacy of indirect screening tests for mastitis can vary in the following descending order: SCC (97.4 %), MCMT (69.6 %) and MWST (63.2 %). In our current investigation, highest (45.7 %) prevalence of bovine SCM was found in southern coastal areas (Chittagong) and lowest (29.6 %) prevalence in industry-based dry areas (Gazipur) of Bangladesh. Higher prevalence of SCM in coastal region may be associated with larger number of lactating cows in the farms, dirty floor condition, cows bathed by pouring water, dirty udder, and overall poor hygienic management which was also reported earlier by Islam et al. (2011). However, our current findings of prevalence of SCM are somewhat lower than the findings of Jha et al. (2010) who reported 55.0 % prevalence of bovine SCM in coastal areas but higher than the findings (20.2 %) of Sarker et al. (2013). In another study, Sharma and Maiti (2010) reported 29.3–78.5 % prevalence of bovine SCM in India.

The overall quarter-wise prevalence of SCM varied along with the different screening tests and SCC. Highest quarter-

Table 2 Effect of different factors on the prevalence of subclinical bovine mastitis

Factors	No. of cows examined	No. of cows infected	Prevalence of subclinical mastitis (%)
Breeds of cows			
Local zebu	71	19	26.8b
Friesian X	62	35	56.4a
Sahiwal	58	25	43.1ab
Red Chittagong	37	12	32.4b
Body condition scores (BCS)			
2.0–2.5	92	51	55.4a
3.0–3.5	65	29	44.5a
>3.5	71	22	30.9b
Age of cows			
3–5	85	31	36.4a
6–8	91	46	50.5b
9–10	52	18	34.6a
Parity of cows			
1–3	97	37	38.1ab
4–6	82	43	52.4a
7–9	49	11	22.4b
Stages of lactation (months after calving)			
1–4	71	34	47.9ab
5–8	34	22	64.7a
9–12	26	7	26.9b
13–16	43	11	25.6b
17–20	49	15	30.6b
Milk yield (L/day)			
1–5	53	15	28.3b
6–10	59	26	44.0ab
11–15	42	22	52.3a
16–20	26	17	65.3a
21–25	43	9	20.9b

Values with the different lowercase Roman letters (a and b) within the same column significantly differ among each other ($P<0.05$)

wise prevalence of SCM was recorded by SCC (55.0 %) followed by CMT (45.7 %), WST (43.5 %), and SFMT (41.2 %). These findings are in agreement with the earlier findings of Sharma et al. (2007) who reported 38.9, 42.0, and 45.0 % prevalence of SCM by MWST, MCMT, and SCC. The position of the quarters, either on individual or combination basis, has also influenced the prevalence of SCM in this study. The rate of SCM indeed remained very high in left front quarters either on individual or combination basis. The higher prevalence of SCM in left front quarters might be due to the fact that no treatment of one quarter, being unnoticed, unclean, or unwashed, could be undertaken or owner's ignorance towards marginal disease in milk production. These findings are also in agreement with the findings of Shahid et al. (2011), who reported that left front quarters were more susceptible to udder infection. Variation in quarter-wise and animal-wise incidence of SCM was also reported in several earlier studies (Sharma et al. 2007; Sudhan et al. 2005), and maximum numbers of animals (74.3 %) were having one quarter infection (Sudhan et al. 2005). Among 892 quarters, 20 (2.2 %) quarters were found blind and could not be subjected to any test for SCM which is similar to the findings of Sharma et al. (2007) and Khan and Muhammad (2005) who reported 1.9 and 8.0 % blind quarters in buffalo and crossbred cows, respectively.

In regard to the breeds of cows, Friesian crossbred evidenced with the highest prevalence (56.4 %), while local zebu has got the lowest prevalence (26.8 %) of SCM in all selected areas of the investigation. The higher prevalence of SCM in Friesian crossbred cows might be due to their pendulous udder position, high milk yield, and wider opening of the teat canal. It is interesting to note that the exotic high yielding breeds of cows are more prone to SCM than local poor yielding breeds. This observation is in agreement with Radostits et al. (2007) who reported significant difference of SCM prevalence among the breeds of animals. In a recent study, Moges et al. (2011) reported that Friesian crossbred cows had higher (71.8 %) rate of udder infection than zebu breeds (28.2 %). However, the differences in

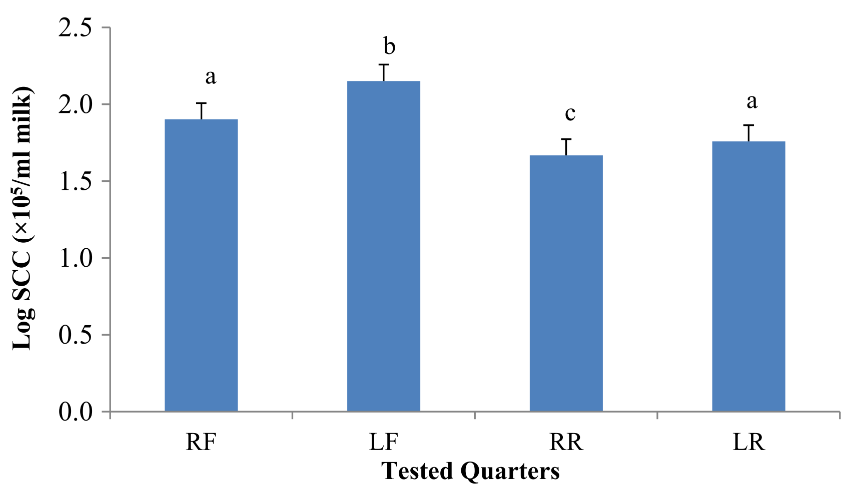

Fig. 3 Milk somatic cell count (Mean±SEM) in each quarter of cows udder with subclinical mastitis. Values within the different quarters (*a*, *b*, *c*) differ significantly

Table 3 Prevalence of subclinical bovine mastitis (animal-wise and quarter-wise) as detected by different screening tests and somatic cell count

SL no.	Tests	No. of cows tested, reacting positive and % positive	Total no. of functional quarters, reacting positive and % positive	Quarter-wise prevalence							
				Individual basis				Combination basis			
				RF	LF	RR	LR	Right side	Left side	Front quarter	Rear quarter
1	CMT	228	892 (20)	224	225	223	220	447	445	449	443
		148	408	107	114	90	97	197	211	221	187
		64.9	45.7	47.8	50.7	40.3	44.0	44.0	47.4	49.2	42.2
2	WST	228	892 (20)	224	225	223	220	447	445	449	443
		138	388	102	110	90	86	192	196	212	176
		60.5	43.5	45.5	48.9	40.3	39.0	42.9	44.0	47.2	39.7
3	SFMT	228	892 (20)	224	225	223	220	447	445	449	443
		132	368	96	103	88	81	184	184	199	169
		57.9	41.2	42.9	45.8	39.5	36.9	41.1	41.3	44.3	38.1
4	SCC	228	892 (20)	224	225	223	220	447	445	449	443
		164	491	124	138	118	111	242	249	262	229
		71.9	55.o	55.3	61.3	52.9	50.4	54.1	56.0	58.3	51.7

Figures in the parentheses indicate the total number of blind quarters

the prevalence of SCM in various breeds might also be due to different managerial systems of the farms in different regions.

Although the prevalence of SCM was found in all stages of lactation but highest percentage (64.7 %) was recorded during the late lactation stage (5 to 8 months). The high rate of SCM in late lactation might be due to cow to cow transmission of contagious pathogens. Higher prevalence in late lactation might also be due to the fact that this period is more vulnerable to udder infection. Our observation agreed with the several independent earlier reports (Patil et al. 1995; Sharma et al. 2007; Jha et al. 2010) in this regard.

We investigated the effect of BCS on the prevalence of SCM and highest (55.4 %) prevalence was recorded in cows having BCS 2.0–2.5. In dairy cows, BCS is one of the important factors affecting the occurrence of SCM (Sarker et al. 2013), and cows having poor BCS are more susceptible to SCM (Jarassaeng et al. 2012). Healthy cows possess decreased susceptibility to SCM may be due to their more effective host defense mechanisms.

The effect of parity on the prevalence of SCM, as recorded in different breeds of cows in this study showed an increasing trend of udder infection with the advancement of parity and highest (52.4 %) prevalence of SCM, was recorded in cows at parity 4–6. However, this trend of udder infection declined in the subsequent parities (lowest at parity 7–9). These findings are supported by Rahman et al. (2009) and Jha et al. (2010) who reported highest prevalence of mastitis at parity 4–7. In another study, Jarassaeng et al. (2012) found significantly lower prevalence of SCM at parity 1–3 of cows.

The daily milk yield and age of the cows also affected the occurrence of SCM in our current study. Cows those yielded 16–20 L milk/day (65.3 %) and of 6–8 years old (50.5 %) were more defenseless to SCM. In earlier studies, Jha et al. (2010) and Siraj et al. (2012) also reported higher prevalence of SCM in cows having higher milk yield, advancing age, and parity. The variability of SCC was also observed according to breeds, BCS, age, parity, lactation stage, and per day milk yield of the cows and mean SCC $>2.0\times10^5$ cells/mL milk

Table 4 Percentage accuracy, sensitivity, and specificity of various indirect tests used for the diagnosis of bovine subclinical mastitis. Data presented as number (%)

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	*k*	*r* (%)
CMT	892	408 (45.7)	307 (78.3)	101 (24.7)	325 (67.1)	159 (32.8)	70.0	65.8	76.2	75.2	0.77	78.2
WST	892	388 (43.5)	271 (69.8)	117 (30.1)	307 (60.4)	197 (39.0)	64.8	57.9	72.4	69.8	0.53	68.3
SFMT	892	368 (41.2)	239 (64.9)	129 (35.0)	295 (56.2)	229 (43.7)	59.9	51.0	69.5	64.9	0.41	56.6
SCC	892	491 (55.0)	455 (92.6)	36 (7.3)	305 (76.0)	96 (23.9)	85.2	82.5	89.4	92.7	0.88	92.4

TP true positive, *FP* false positive, *TN* true negative, *FN* false negative, *PPV* positive predictive value, *k* Kappa index (% agreement), *r* Pearson's correlation samples were used to describe the status of quarter infection with SCM. The mean SCC was found higher in the front quarters than the rear quarters. The left front quarters were mostly affected with SCM and had an average SCC of 8.6×10^5 cells/mL milk sample; these findings are also supported by dos Reis et al. (2011).

The worth of the diagnostic test depends on its accuracy, sensitivity, specificity, predictive values, percentage agreement, and field applicability. In this study, CMT was found to be more accurate, sensitive, and specific than the other two field tests (WST and SFMT) after the laboratory diagnostic test like SCC. The highest CMT scores (50.7 %) were observed in the left front quarters which coincides the results of SCC. Barbosa et al. (2002) reported that the SCC and CMT were dependent and highly correlated for diagnosis of SCM. The correlation between higher CMT scores and elevated trends of SCC in the milk samples of positive quarters was clearly demonstrated in this study. Thus, CMT exhibited excellent agreement with the findings of milk SCC, while other two screening tests (WST and SFMT) had fair to good agreement with the results of SCC in our present study.

In conclusion, although different screening tests are available to diagnose both clinical and subclinical types of mastitis in dairy animals at field conditions, their diagnostic accuracy, sensitivity, and specificity vary greatly. Therefore, the use of any single test may not be reliable in diagnosing SCM. Our current investigation indicates that the result of CMT supported by SCC might be used effectively for pinpoint diagnosis of SCM in dairy animals, than alone.

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Conflict of interest The authors of this manuscript hereby declare that they have no conflict of interest regarding the publication of this research work.

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