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PREVALENCE AND RISK FACTORS OF SUB-CLINICAL MASTITIS IN LACTATING BLACK BENGAL GOATS DETECTED BY USING INDIRECT AND DIRECT METHODS OF SOMATIC CELL COUNT IN BANGLADESH

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ABSTRACT

Background: Sub-clinical mastitis (SCM) has been reported to be more widely prevalent than clinical mastitis (CM) in lactating dairy animals and associated with heavy economic losses with changes in quality and quantities of milk worldwide. Several indirect and direct methods of Somatic cell count (SCC) are used to detect SCM in dairy animals but reports on their comparative evaluation are very limited in inland literature especially in goats.

Objective: This study was undertaken to evaluate the comparative efficacy of different indirect tests with direct microscopic milk SCC (DMMSCC) to detect the prevalence of SCM in lactating goats of smallholder and organized goat farms with their associated risk factors in Bangladesh

Materials and Methods: Milk samples were collected aseptically from both halves (n = 140) of each of 70 apparently healthy lactating Black Bengal goats (BBG) at different stages of lactation from Rajshahi Goat Development Farm (RGDF; n = 20) and smallholder farms (n = 50) of adjacent villages of the Bangladesh Agricultural University (BAU) campus, Mymensingh during the period from July 2010 to June 2011. The White side test (WST), Surf field mastitis test (SFMT), California mastitis test (CMT) and DMMSCC were used to diagnose the SCM in milk samples as per instructions of the diagnostic methods. The potential risk factors associated with the prevalence of SCM were analyzed using multiple regression and uni-variable logistic regression analysis.

Results: The overall an average of 30.0% prevalence of SCM was recorded in this study irrespective of the method used. The comparative evaluation of four milk screening tests for SCM showed higher efficacy with the WST (32.0%) and CMT (31.43%) in comparison to SFMT (28.0%) and DMMSCC (26.0%). The significantly higher prevalence of SCM was recorded in late lactation (37.90%), long teat (44.40%) and shortest teat end to floor distance (33.30%) in lactating goats. Uni-variable logistic regression analysis depicted that SCM was more prevalent in does with increased age, parity and during winter season.

Conclusions: This study recorded comparatively higher efficacy with WST and CMT in comparison to SFMT and DMMSCC to detect SCM in lactating goats. It may be concluded that either WST or CMT along with bacterial culture of milk samples are required for accurate diagnosis of SCM in goats. Moreover, it may also be suggested to test the milk samples simultaneously of both the haves of lactating goats by using a single test to compare their results between the half for the detection of SCM. Some risk factors are found associated with the prevalence of SCM in goats and therefore, effective measures need to be required to improve these risk factors in both goat development farm and smallholder farm levels to control caprine mastitis in Bangladesh.

Keywords: Black Bengal goats, Sub-clinical mastitis, Prevalence, Indirect tests, Direct somatic cell count, Goat farm, Smallholder farmers, Risk factors

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INTRODUCTION

The term 'mastitis' comes from the Greek words: 'mastos' (for breast) and 'itis' (for inflammation. Mastitis is the inflammation of the mammary gland, including not only intra-mammary tissues of the mammary gland but also related anatomical structures.[1] The udder of goat is consisted of two halves, each one has single mammary unit which include the mammary glandular parenchyma, lactiferous ducts and sinus and teat canal ended by a teat orifice.[2] Mastitis is of importance from three perspectives: (a) economic (mortality of animals, treatment costs, reduced quantity and quality of milk), (b) hygiene (the risk of infection or poisoning of consumers by consuming infected milk) and (c) legal (definitions of bacteriological milk quality).[3] The first publication on mastitis of a dairy animal was on mastitis in goats[4] but the more recent times, the vast majority of mastitis publications dealt with mastitis in cows.[5] The prevalence of CM is very low in goats[6,7] whereas the high prevalence of SCM goes undiagnosed and often ignored because of smallholder farmers are usually reared goats mainly for meat rather than milk especially Black Bengal goats, moreover there is unfamiliarity with the interpretation of the results of the available indirect diagnostic tests for SCM. The same diagnostic tests are commonly used in cows as well as in goats for diagnosis and monitoring of udder health problems. However, the prevalence of CM in goats is usually below 5.0%[6,8] while SCM ranges from 9 to 50%.[9,10] The intra-mammary infection (IMI) may result an inflammation (mastitis) which can be CM or SCM. The CM is characterized by classical signs of inflammation with pain, swelling, erythema, warmth of the udder with decreased milk yield whereas the SCM can be defined as an infection of the udder gland without the visible signs. SCM is characterized by reduced milk production, increased SCC and bacterial presence in the milk but it lacks the macroscopic changes typically of the clinical stage. Diagnosis of CM can be based on the appearance of abnormality of milk, udder and occasionally health of lactating animals but the diagnosis of SCM is more problematic since the milk, udder and health of lactating animals appears apparently normal but usually has an elevated MSCC and the majority of these cells are inflammatory cells and thus the MSCC represents the inflammatory response of the udder gland to an invading pathogen. Diagnosis of SCM can be made either direct or indirect measurement of MSCC and isolation and identification of mastitis pathogens in milk.[11-13] The prevalence of CM is much higher in cows than in goats, whereas MSCC is much higher in goats than in cows.[14,15] Although SCC is not a perfect test for IMI in cows, it is currently the most frequently used tool to monitor udder health in cows.[5] Some authors have reported that MSCC have an unreliable indication of SCM,[16] SCC and CMT are of limited value for goats[17] and the relationship between IMI and MSCC has not been always correlated.[12,18] Large number of SCC have been recorded in milk from infected and also from culture-negative goats and SCC seems to be affected by infectious as well as non-infectious factors.[5,12,19] Accordingly, the use of MSCC as a diagnostic test for the diagnosis of SCM in goats has been questioned.[5] The goat flock level-risk factors including flock size, rearing system, floor condition and milking methods while host-level factors include age, breed, parity, litter size, stage of lactation, teat lesions and teat end shape have been reported to have a significant effect on the prevalence of mastitis.[20-23] The bacteria isolated and identified from milk of dairy lactating cattle with CM (*Staphylococcus* spp., *Staphylococcus aureus*,

Streptococcus spp., *Bacillus* spp. and *Escherichia coli*)[24,25] and SCM (*S. aureus*, *E. coli*, *Enterobacter* spp., Coagulase-Negative Staphylococcus (CNS), *Bacillus* spp., *Pseudomonas aeruginosa*);[26,27] buffaloes with SCM (*Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp. and *E. coli*)[28] and goats with CM (CNS, *Staphylococcus* spp., *S. aureus*, *Streptococcus* spp., *E. coli*, *Pseudomonas*, *Klebsiella* spp., *Bacillus* spp.)[20,29,30] and SCM (CNS, *Staphylococcus* spp., *S. aureus*, *Streptococcus* spp., *Bacillus* spp., *E. coli*, *Pseudomonas*, *Klebsiella* spp.,)[20,29,30] from Bangladesh. Caprine mastitis can be caused by a number of pathogens but the most important bacterial genus is *Staphylococcus*, usually divided into *S. aureus* and CNS. The CNS are generally the most prevalent and can cause persistent infections that result in increased cell counts and low-grade mastitis with some recurring clinical episodes.[20,31-33] It appears that the same bacteria have been implicated as causal agents of both the clinical and subclinical forms of mastitis in both small and large ruminants. No significant difference on the types of bacterial pathogens between clinically (93.22%) and sub-clinically (95.92%) affected quarters has been reported in does in Bangladesh.[29] SCM usually precedes the clinical form and constitutes a reservoir of pathogens which act as a source of infection to the healthy animals.[34] Currently, goat (26.1 million) has been shown to be the highest population in comparison to cattle (24.086 million) among total ruminants (55.139 million) species in Bangladesh[35] which are mainly reared especially BBG for meat purposes but its health management status especially mastitis aspect has been neglected and ignored, however some unplanned and scattered research findings on caprine mastitis have been published from Bangladesh.[36,37] This paper describes to evaluate the comparative efficacy of different indirect tests with DMMSCC to detect the prevalence of SCM in lactating goats of smallholder and organized goat farms with their associated risk factors in Bangladesh

MATERIALS AND METHODS

Each udder and teat of all the available lactating Black Bengal goats (BBG) of the Rajshahi Goat Development Farm (RGDF) and adjacent villages of the Bangladesh Agricultural University (BAU) campus, Mymensingh were examined by manual palpation to detect any anatomical and clinical abnormalities during the period from July 2010 to June 2011. Milk was examined for discoloration, clots or flakes, pus, blood staining and consistency. A total of 70 lactating goats with 140 udder halves were found apparently healthy on physical palpation of udder and visual milk examination which were selected for this study.

Milk samples were collected aseptically from both halves (n = 140) of each of 70 apparently healthy lactating goats at different stages of lactation from RGDF (n = 20) and smallholder farms (n = 50) of adjacent villages of the BAU campus. Briefly, the teats were wiped with swabs soaked in 70% ethanol and thereafter, few streams of milk were discarded. Then, 10 to 15 ml of milk samples was collected into a sterile tube, labeled and immediately brought to the laboratory. The samples were kept at 4 ^{0}C and immediately tested for SCM.

Milk samples collected from the goat farm were tested immediately by using indirect field tests and samples collected from smallholder farms were brought to laboratory immediately and then tested with both the indirect and direct (SSC) methods of diagnosis of SCM.

White side test (WST)

The WST was used as per method[38] and briefly described in our earlier report.[39] Each milk sample was thoroughly mixed carefully to avoid violent shaking. Then 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% sodium hydroxide) 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 grading of the reaction was considered as 0 (negative), 1+ (weak +ve), 2+ (distinct) and 3+ (strong +ve).

Surf Field Mastitis Test (SFMT)

Reagent solution for SFMT was composed of 3.0% household detergent (Surf-excel, Lever Brother Bangladesh) and the test was performed and scored following the method described earlier.[40] A shallow half black paddle having four cups was used and was rinsed after each use. Briefly, 2.0 ml of milk was drawn from the bottle into test cup and an estimated 2.0 ml reagent (commercial Surf-excel 3.0% in distilled water) was squirted from a polyethylene wash bottle. Mixing was accompanied 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+.

California Mastitis Test (CMT)

The CMT was performed by using the CMT kit (Leucocytest® Synbiotics Corporation-2, Lyon, France) as per kit manufacturer instruction. Briefly, a shallow half black paddle having four cups was used and was rinsed after each use. About 2.0 ml of milk sample was drawn from bottle into the CMT paddle and equal volume of CMT reagent was mixed in each cup immediately by swirling / circular motion for few seconds and the reaction was graded by intensity of gel formation and color change within 30 seconds. The test reaction was graded as, 0 (negative), 1+ (weak +ve), 2+ (distinct +ve) and 3+ (strong +ve).

Milk somatic cell count (MSCC)

The Direct microscopic MSCC (DMMSCC) was performed as per method described earlier.[39,41,42] Briefly, fresh milk was collected and was mixed thoroughly and the cream was dispersed throughout the specimens. A uniform smear over the pre-drawn one square centimeter (1 cm^2 = 1 cm × 1 cm) area of a degreased microscopic slide was made by using 10 µl of milk. The milk film was allowed to air dry in a horizontal position. The air dried milk film stained with Broadhurst-Paley stain[43] in triple step procedure by firstly, immersing the slides in xylene for two minutes then drain dried; secondly, the slides were immersed in 95% ethyl alcohol for 2-5 minutes then again drain dried (to remove fat) and finally, the slides were immersed in the Broadhurst-Paley stain for 5 seconds and briefly rinsed with water and drain dried. Then the slides were examined under an oil immersion objective in routine milk analysis, i.e. counting the number of cells in 25 fields using a working factor of 20,000. In stained slides, milk solids stained pink, mononuclear cells are deep blue, PMN leukocytes are pale blue and

bacteria are light to deep blue colored. The total number of cells counted is multiplied by the working factor of the particular microscope to obtain the number of cells / ml of milk.[44] The results of SSC was recorded by counting of somatic cells matching the scores of the CMT and the results were graded as Trace (negative / normal), 1+ (weak +ve), 2+ (distinct +ve) and 3+ (strong +ve) which were considered as indicators of SCM (Table 1).

Table 1. Comparison and interpretation of CMT and MSCC scores in goat milk[41]

CMT (California Mastitis Test) results			MSCC (Milk somatic cell count) results	
Grade/ Score	Reaction	Av leukocytes / ml	MSCC/ml	Interpretation
0	No reaction	68,000	< 1,000	Healthy udder
Trace	Slight slime	268,000	2,000-50,000	Infection by weak pathogens
1+	Distinct slime	800,000	>1,500,000	Signal of infection
2+	Gel formation	2560,000		
3+	Strong gel formation	≥10,000,000		

Risk factors analysis

A structured questionnaire was used to collect the host, management and environmental factors to detect their influence on the prevalence of SCM in lactating goats.

Statistical analysis

The percentage accuracy of the tests and sensitivity, specificity and predictive values of the CMT, WST, SFMT and SCC 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 different risk factors by using Statistical Package for Social Science (SPSS) version 17.0

RESULTS

An attempt was made to evaluate the efficacy of different indirect tests and direct MSCC to compare the prevalence of SCM between farm and rural smallholder management lactating goats. The udder and teats of all the available lactating goats of the RGDF and smallholder farmers were thoroughly examined on the method of palpation, and only 70 lactating goats (20 goats of the RGDF and 50 smallholder goats) were found apparently healthy which were selected for this study. A total of 140 milk samples of 70 lactating goats were examined visually and by three indirect (CMT, WST and SFMT) and direct MSCC tests. However, the CMT was used for 140 milk samples, but WST, SFMT and SCC were used in 100 milk samples (Table 2).

Animal and test-wise overall prevalence of SCM was recorded in 32.0% with WST, 28.0% with SFMT, 31.43% with CMT and 26.0% with SCC tests (Table 2). Out of 140 udder halves tested with indirect and direct tests, of which 40 (28.57%) were affected with SCM. There was no difference on the prevalence rates of SCM between right and left halves in lactating BBGs

(Table 2). Table 2 shows the comparative efficacy of different indirect and direct SCC tests used for the detection of SCM in goats with similar efficacy with WST (32.0%) and CMT (31.43%), followed by SFMT (28.0%) and lowest with MSCC (26.0%).

Table 2. Comparative efficacy of indirect and direct tests for the detection of sub-clinical mastitis in Black Bengal lactating goats

S/N	Tests used	Udder halves	Total No. tested	Positive, No. (%) 1+	2+	3+	Overall No. (%)
1.	White Side Test (WST)	RUH	50	09 (18.00)	07 (14.00)	0	16 (32.00)
		LUH	50	09 (18.00)	07 (14.00)	0	16 (32.00)
		Total	100	18 (18.00)	14 (14.00)	0	32 (32.00)
2.	Surf Field Mastitis Tests (SFMT)	RUH	50	10 (20.00)	04 (08.00)	0	14 (28.00)
		LUH	50	10 (20.00)	04 (08.00)	0	14 (28.00)
		Total	100	20 (20.00)	08 (08.00)	0	28 (28.00)
3.	California Mastitis Test (CMT)	RUH	70	12 (17.14)	10 (14.29)	0	22 (31.43)
		LUH	70	12 (17.14)	10 (14.29)	0	22 (31.43)
		Total	140	24 (17.14)	20 (14.29)	0	44 (31.43)
4.	Somatic Cell Count (SCC)	RUH	50	05 (10.00)	06 (12.00)	2 (4.00)	13 (26.00)
		LUH	50	05 (10.00)	06 (12.00)	2 (4.00)	13 (26.00)
		Total	100	10 (10.00)	12 (12.00)	4 (4.00)	13 (26.00)

RUH = Right udder halves LUH = Left udder halves

The reports on the risk factors associated with the prevalence of SCM in lactating goats are reviewed and compared with findings of the present study (Table 3). Highest prevalence of SCM was recorded at late (37.90%), followed by mid (28.10%) and lowest in early (11.10%) lactation (Table 3). Highest prevalence of SCM was found in longest teat length (44.40%) in comparison with medium (28.10%) and short (27.60%) teat length (Table 3). Similarly, lactating goats divided into four groups based on teat end to floor distance (cm) showed significantly ($p < 0.01$) highest prevalence of SCM in lactating goats with short distance (9 cm) between teat end and floor (33.30%) in comparison to 10 cm (14.60%) and 11 cm (16.70%) whereas none of the goats had SCM with 12 cm distance (Table 3). Out of 70 goats examined, comparatively higher prevalence of SCM was recorded in goats had pointed (50.0%) than rounded (25.90%) teat tips (Table 3). Of the four lactating goats had supernumerary teat, of which two (50.0%) affected with SCM and of the 66 lactating goats with scabies teat lesions, 19 (28.80%) were affected with SCM (Table 3). However, there was no influence of udder size and teat diameter on the prevalence of SCM in goats (Table 3).

Table 3. Risk factors associated with SCM in Black Bengal goats in Bangladesh

S/N	Risk factors	Sub-factors	No. of does tested	Positive No. (%)	Islam et al.[20] No. of does	Positive No. (%)	Razi et al.[21] No. of does	Positive No. (%)	Ferdous et al.[30] No. of does	Positive No. (%)
1.	Age (years)	2	-	-	021	00	-	-	-	-
		3	-	-	072	16 (22.22)	34[a]	04 (12.50)	37[a]	13 (35.14)
		4	-	-	124	61 (49.19)	20[b]	05 (25.00)	57[b]	24 (42.11)
		5	-	-	025	13 (52.00)	05[c]	04 (80.00)	26[c]	15 (57.69)
	Total	-	-	-	242	90 (37.19)	59	13 (22.03)	120	52 (43.33)
2.	Rearing system	Farm	20	05 (25.00)	216	83 (38.43)	26	03 (11.53)	-	-
		Rural	50	16 (32.00)	026	07 (26.92)	33	08 (24.24)	-	-
		Total	70	21 (30.00)	242	90 (37.19)	59	13 (22.03)	-	-
3.	Parity	1st	-	-	020	0	09	01 (11.11)	11	0
		2nd	-	-	036	02 (05.56)	21	01 (04.76)	22	05 (22.73)
		3rd	-	-	047	17 (36.17)	16	01 (06.25)	19	10 (52.63)
		4th	-	-	062	33 (53.22)	07	04 (57.14)	35	17 (48.57)
		5th	-	-	061	33 (54.09)	03	02 (66.66)	33	20 (60.61)
		6th	-	-	011	09 (81.82)	03	02 (66.66)	-	-
	Total		-	-	242	90 (37.19)	59	13 (22.03)	120	52 (43.33)
4.	Litter size	One	-	-	012	02 (16.67)	-	-	15	02 (13.33)
		Two	-	-	196	68 (34.69)	-	-	74	30 (40.54)
		Three	-	-	026	15 (57.69)	-	-	31	20 (64.52)
		Four	-	-	008	05 (62.50)	-	-	-	-
		Total	-	-	242	90 (37.19)	59	13 (22.03)	120	52 (43.33)
5.	Lactation stage (months)	Early (<3)	09	01 (11.10)	153[d]	71 (46.41)	55[g]	09 (16.37)	71	32 (45.07)
		Mid (3-4)	32	09 (28.10)	050[e]	14 (28.00)	03[h]	01 (33.33)	27	12 (44.440
		Late (>4)	29	11 (37.90)	039[f]	05 (12.82)	01[i]	01 (100)	22	08 (36.36)
	Total		70	21 (30.00)	242	90 (37.19)	59	13 (22.03)	120	52 (43.33)
6.	Teat lesions	Present	-	-	078	66 (84.62)	-	-	38	18 (47.39)
		Absent	-	-	164	24 (14.63)	-	-	82	34 (41.46)
	Total		-	-	242	90 (37.19)	59	13 (22.03)	120	52 (43.33)
7.	Type of housing floor	Slatted	-	-	-	-	22	03 (13.63)	-	-
		Concrete	-	-	-	-	04	0	-	-
		Earthen	-	-	-	-	33	08 (24.24)	-	-
	Total		-	-	242	90 (37.19)	-	-	-	-
8.	Udder size (cm)	10-11	34	10 (29.40)	-	-	-	-	-	-
		>11-12	36	11 (30.60)	-	-	-	-	-	-
	Total		70	21 (30.00)	-	-	-	-	-	-
9.	Teat length	4.0 cm	29	08 (27.60)	-	-	-	-	-	-
		5.0 cm	32	09 (28.10)	-	-	-	-	-	-
		6.0 cm	09	04 (44.40)	-	-	-	-	-	-
	Total		70	21 (30.00)	-	-	-	-	-	- Contd.

10.TEFD	09 cm	36	12 (33.30)	-	-	-	-	-	-
	10 cm	24	07 (14.70)	-	-	-	-	-	-
	11 cm	06	02 (16.70)	-	-	-	-	-	-
	12 cm	04	0	-	-	-	-	-	-
Total		70	21 (30.00)	-	-	-	-	-	-
11.Teat diameter	1.0 cm	16	05 (31.30)	-	-	-	-	-	-
	2.0 cm	54	16 (29.60)	-	-	-	-	-	-
Total		70	21 (30.00)						
12.STT	Rounded	58	15 (25.90)	-	-	-	-	-	-
	Pointed	12	06 (50.00)	-	-	-	-	-	-
Total		70	21 (30.00)	-	-	-	-	-	-
13.Teat conditions	SNT	04	02 (50.00)	-	-	-	-	-	-
	Scabies	66	19 (28.80)	-	-	-	-	-	-
Total		70	21 (30.00)	-	-	-	-	-	-

[a]Age 2-3 years [b]Age >3-4 years [c]Age >4-5 years [d]Early (<2 months) [e]Mid (2-3 months)
[f]Late (>3 months) [g]1-2 months [h]>2-3 months [i]>3-4 months
TEFD = Teat end to floor distance STT = Shape of teat tips SNT = Supernumerary teat

DISCUSSION

Mastitis is a complex economic disease of dairy animals that generally involves interplay between management and infectious agents, having different degrees of intensity and variation and residual effects.[5,6,45-49] Mastitis is primarily classified into clinical (per-acute, acute, sub-acute, chronic & gangrenous) and sub-clinical forms.[6,7,30,37,49] Mastitis is usually incriminated with multifarious agents including bacterial, mycoplasma, yeast and other fungi.[20,44] SCM is characterized by normal appearance of milk with no visible abnormalities in the mammary tissues of the affected animals. The SCM is important due to (a) it is 15 to 40 times more prevalent than CM and causes great economic losses than CM, (b) it usually gradually precedes the CM, (c) it is of long duration, (d) it is difficult to detect, (e) it reduces milk production and (f) it adversely affects milk quality.[50-55] It negatively influences the quantitative and qualitative parameters of milk and mastitic milk is a source of infection to both susceptible suckling animals and the consumer and therefore a direct threat to human and animal health.[13] SCM is one of the most challenging diseases in dairy animals including goats because it has been linked to production loss, downgrading of milk quality and hygiene, increased replacement cost and considerable veterinary expenses.[45] Timely and accurate diagnosis of intra-mammary infection in lactating dairy animals, especially SCM is required for the treatment, prevention and control of mastitis.

The diagnostic tests for SCM have been divided into general (phenotypic) and specific (genotypic). The phenotypic tests are those that identify general alteration in the milk which are not specific to any pathogen, whereas genotypic are specific which include specific culture and molecular tests for confirmation of specific causative agents.[25,56] The CMT, WST and SFMT have been most widely used field indirect diagnostic tests, whereas MSCC, culture and

isolation of the causative agents have been used as direct laboratory diagnostic methods of SCM.[13,54]

Primarily leukocytes (macrophages, polymorphonuclear neutrophils (PMN) and lymphocytes) are the important cellular components naturally present in milk are considered as milk somatic cells (MSC). In addition to leukocytes, epithelial cells (EC) and cytoplasmic masses (CMs) are also present in milk. These leukocytes are always small quantities of immune cells that enter the milk compartment of the udder and their function is to protect the udder against mainly bacterial infection. The bacterial infection is usually caused to increase these cells in milk which is a good indicator of intra-mammary infection (IMI). The MSCC is used to identify lactating animals likely to have an IMI with bacteria, especially to detect udder health and milk quality[57] as well as the level or occurrence of SCM in lactating animals.[58] However, the MSCC is influenced by many factors including animal species, breed, milk production level, lactation stage, genetics, parity, day-to-day variation, diurnal variation, milking interval, time of sampling, sampling procedures, stress and trauma, environmental factors, seasonal and management practices.[59] Though MSCC is subjected to variation, it is still used as an indicator of milk quality and udder health, and when MSCC > 2×10^5 cells / ml, the udder of the cow is considered to be infected and when MSCC > 4×10^5 cells / ml the milk is deemed unfit for human consumption in the European Union (EU).[60,61] For caprine and ovine milk, the cut-off value is 1 x 10^6 cells / ml in the USA.[57] Most milk processing companies with use of bulk milk somatic cell count (BMSCC) to estimate herd mastitis prevalence but this relationship is more complex in goats.[62]

The MSCC seems to be affected by infectious and non-infectious factors in goats.[11] Several studies have shown that non-infectious factors also influence MSCC in goats such as stage of lactation, e.g. the higher the SCC the later the stage of lactation,[19,63,64] parity,[65,66] estrus[67] and breed.[68] In addition, a high MSCC in goats is not always accompanied with a positive bacterial culture.[32] Both indirect and direct SCC methods are commonly used to detect SCM in animals. MSCC can be measured at either the gland level (the udder), individual animal level and herd level (bulk milk SCC).[11]

The overall 30.0% prevalence of SCM recorded in this study in lactating goats, however both higher and lower prevalence of SCM have been reported earlier in inland reports. The overall higher prevalence of SCM in lactating goats have been reported as 37.19% from the districts of Mymensingh, Rajshahi and Dhaka by using multiple indirect tests,[20] 36.0% from Savar, Dhaka by using CFT,[69] 38.75% by CFT in Dinajpur,[30] 35.0% in Barishal by using multiple indirect tests[22] and 50.9% by CFT in Chittagong.[23] The lower prevalence of SCM has also been reported as 18.64% by CMT in Mymensingh.[21] However, the prevalence of CM in lactating goats has been reported at a very low rate in comparison to SCM in Bangladesh[37] and elsewhere.[5,50,51] The analysis of 30 reports mainly published based on hospital data showed an overall of 2.5% prevalence of CM in goats in Bangladesh[37] which supports the 3.3% prevalence of CM based on analysis of the hospital cases in Chittagong.[8] However, comparatively higher prevalence of CM have been reported in goats based on research findings and some of which are 4.54% in Mymensingh, Rajshahi and Dhaka,[20] 5.27% in Mymensingh and Jaipurhat,[29] 11.67% in Dinajpur[30] and 6.0% in Mymesningh.[55] These variations might be due to access of

all the CM cases during research but all CM cases are usually not brought for treatment to the veterinary hospitals in Bangladesh. These variations on the prevalence of mastitis could be due to the differences in breeds, animal husbandry practices and environmental factors which influence intra-mammary infection in goats.[20]

Modified White Side Test (MWST)

The MWST as described by Murphy and Hanson in early of 1941 was used to detect evidence of mastitis in a single quarter of a cow's udder.[70] The WST[71] as originally described was performed by adding 2.0 ml 1N NaOH to 10 ml of foremilk in a watch glass and then beating the mixture with a glass rod. A positive reaction was described as the formation of a 'viscid mass.' Murphy observed that in cases where the typical viscid mass was not in evidence, many of the milk-NaOH mixtures contained a precipitate which varied in amount and appearance. The modification consisted of using one drop of 1N NaOH to five drops of milk and mixing for 20 seconds on a glass plate with a dark background. Reactions were graded according to the amount of precipitate and the degree of opacity. The WST modified in this manner was found to parallel closely the SCC in ability to detect udder infection and SCM.[72] The WST has detected 32.0% prevalence of SCM in this study which is comparatively lower than the 38.96%[55] and 35.0%[22] have been reported in lactating goats with WST in earlier inland reports. The WST is also an indicator field test for the detection of SCM in lactating animals. Leucocytes nuclei are mainly responsible for the formation of the precipitate in the WST reaction and calcium chloride dispersed the precipitate formed by the leukocyte nuclei.[73]

California Mastitis Test (CMT)

The CMT was introduced in 1957,[74] remains as an effective and rapid 'animal side' test that identifies infected quarters in dairy large and small ruminants especially diagnosis of SCM worldwide.[46,56,75] The principle of this test is based on disrupting the membranes of somatic cells by the CMT reagent (sodium lauryl sulfate) and the reaction of their DNA and proteins contained in the cells and subsequent formation of gel which relates to the number of somatic cells in the milk. This is how the CMT gives an indication of the somatic cells in the milk sample to detect SCM.[17,76] The CMT score is an indirect measure of SCC which is well correlated with SCC in goats.[77] Therefore, the CMT is a crude test for SCC in milk should be used with causation in goats.

To perform the CMT, equal amounts of milk and CMT reagent (sodium lauryl sulfate) are added to individual wells of the CMT paddle and swirled while scoring. The paddle are made of white plastic that allow for easy visualization of 'stringing' with even small changes in viscosity. Coagulation (gel formation) of the milk and color change indicates the presence of infection. The score in common use ranks the samples from 0, 1+, 2+ and 3+ (1+ (trace) to 3+ positive). Concurrent to evaluate the change in viscosity, the CMT reagent also contains a pH indicator that will turn from blue to yellow in acidic milk.

The CMT has detected an overall 31.43% prevalence of SCM in goats which is comparatively lower than 39.83%[55] and 35.0%[22] have been report in lactating goats with CMT from Bangladesh. However, the interpretation of the CMT results in goats is more complicated than

in cows because of the presence of higher SCC in goats than cows. For this reason the CMT may be best used to evaluate trends in animals or to compare the results for one half of the udder with those for the other half.

Surf Field mastitis test (SFMT)

The principle of the SFMT is that when 3.0% household detergent is added into milk sample containing bacterial load gives positive result by thickening the surface of milk and it also causes rupture of somatic cell and release DNA and other cell contents.[78] DNA and detergents unite to form a gel, consistency of gel depends upon the number of somatic cells. More cells more thick gel and vice versa. The SFMT score is made as 1+ = Moderate, 2+ = Severe, 3+ = More severe and 4+ = Very severe.[40] This study has detected 28.0% positive for SCM in goats which is also lower than 38.10%[55] and 30%[22] have been reported in lactating goats with SFMT earlier inland reports. The SFMT is used for screening of milk samples initially. However, due to incorrect execution, the usability of the test remains questionable.[79]

Milk somatic cell count (MSCC)

The MSCC is commonly used to monitor udder health and diagnosis of subclinical intra-mammary infection (IMI) in lactating animals. The MSCC is defined as the concentration of leukocytes (75% includes neutrophils, lymphocytes, macrophages, erythrocytes) and epithelial cells (25%) in milk and is expressed as 'cells per ml of milk.'[58,80] Leukocytes are present to facilitate the removal of invading pathogens and epithelial cells are continuously shed from glandular tissue into milk. As a consequence, healthy quarters without IMI have a SCC ranging from 10,000 to 100,000 cells / ml and SCC of 200,000 / ml should indicate mastitis.[81] In the presence of IMI, leukocytes are recruited to move from the circulation into milk, resulting in an increased SCC.[82] The oldest method for enumerating SC in milk is direct microscopic counting, often combined with methylene blue staining, which although slow and labor-intensive, remains in many instances the reference method against which other methods are calibrated.[83]

The SCC in bovine milk has been reported in healthy (< 2.0 × 10^5 cells / ml), SCM (3-5 × 10^5 cells / ml) and CM (> 5 × 10^5 cells / ml) affected lactating cows.[83.84] The best SCC threshold for defining caprine SCM has been reported as 500 × 10^3 cells / ml of milk but this threshold had a poor predictive value (28.5%) and only 62.3% of samples were correctly classified.[77] The goats having SCC ≥ 1.0 million per ml of milk were considered as positive for SCM.[50,51] A cut-off value of 1,500 × 10^3 cells / ml has been reported to be useful screening tool for detection of *S. aureus* in dairy goats.[63] Most of the MSCC thresholds used to identify IMI in goats range from 500 to 1000 × 10^3 cells / ml.[9,77,85-87] However, a threshold of 345 × 10^3 cell / ml has been proposed to differentiate between infected and non-infected glands in goats.[54]

This study recorded 26.0% prevalence of SCM in lactating goats by using direct microscopic MSCC ≥ 1,500,000 cells / ml whereas CMT detected 31.43% SCM considering SCC ≥ 800,000 cells / ml of milk. These results could not be compared due to lack of similar reports on lactating goats in the inland literature.[36,37] However, the direct microscopic MSCC has successfully been used for the diagnosis of SCM in lactating dairy cows and reported 66.67%

prevalence of SCM with SCC and suggested that SCC method could be the most accurate after cultural isolation, however, the highest prevalence of SCM has been reported with CMT (72.07%) and WST (64.86%) and lowest with SFMT (61.26%) in lactating dairy cows in Bangladesh.[39] A NucleoCounter® SCC-100™ (Counter Electronic- ChemometecA/S, Denmark) has also been used to detect SCM with > 100 × 10^3 cells / ml of milk of lactating dairy cows in Bangladesh with highest prevalence of 55.0% SCM with SCC in comparison to CMT (45.7%), WST (43.5%) and SFMT (41.2%) and concluded that CMT to be the most accurate field diagnostic test after laboratory SCC test.[88] A relationship between SCC and different prevalent bacteria in milk has also been reported with the use of same electronic counter in lactating cows especially with the major pathogens (380.72 × 10^3 cells / ml) which induced higher SCC than minor pathogens (182.67 × 10^3 cells/ ml) but 10% SCM detected quarters based on SCC had no infection.[12]

Electronic cell counters cannot accurately differentiate between epithelial cells (EC), cytoplasmic masses (CMs) and leukocytes. Consequently, when EC and/or CMs are present in high concentrations, cell counts may be artificially elevated if enumerated by electronic cell counters. Differential staining of milk samples is required for conformation of SCC and only nucleated cells (leukocytes) are counted thus yielding a more accurate measure of the SCC of goat milk.[50,51] Therefore, SCM detectable by monitoring of SCC, need careful interpretation due to the higher rate of EC sloughing and the presence of CMs masses in goat milk. The MSCC for goats free from IMI ranges from 270 to 2,000 × 10^3 / ml, EC averages 23 ± 4 ×10^3 cells/ ml and cytoplasm particles averages 150 × 10^3 / ml and therefore, to obtain accurate MSCC for goats, only cell counting procedures specific for DNA should be used.[62,89]

The SCC is a proven tool in SCM diagnosis in dairy cattle but its use in goats is still not widely recognized as a standard diagnostic test because of the biological specifics of these animals.[13,87,90] There are many factors that influence the SCC without an inflammatory reaction which include lactation stage, estrus, breed, milking with respect to mastitis monitoring and diagnosis.[13] The SCC has been reported to be varied from 1.2 × 10^6 to 1.6 × 10^6 / ml in bulk milk tank samples from Spain, France and Italy over a 5 years period.[6] It has also been reported the milk SCC as 779 × 10^3/ ml in 1400 goats,[91] more than 10 million / ml milk[66] whereas it is still not accepted reference range for SCC in goats in EU.[13] However, a threshold SCC of 500 × 10^3 / ml has been reported to distinguish between normal physiological levels and SCM[92] and higher of this level of SCC (> 400 × 10^4 / ml) has been suggested for diagnosis of SCM in dairy goat herds.[93,94]

There are substantial differences among cows, ewes and does in terms of diagnosis of SCM based on milk SCC. The differences result mainly from the fact that in uninfected halves of goat udders there is a high apocrine component of goat milk secretion and a large number of non-infectious factors that can increase MSCC.[68] The apocrine milk secretion in goats, compared to mesocrine secretion in cows, causes MSCC for goats naturally higher than MSCC for cows. An association between MSCC and pathogenic bacteria can contribute to a better understanding of the pathogenesis of SCM in goats. The increased SCC in milk in cows and sheep due to the stage of lactation and parity is mainly caused by IMI. However, when IMI in goats increase MSCC, other non-infectious factors such as estrus, season of milking, milk

yield and stage of lactation also cause an increase in MSCC.[68,95] So, the non-infectious factors cause difficulties for the dairyman to maintain a level of 1,000,000 cells/ ml in goat milk. These non-infectious factors should be taken into account when determining the legal limit for MSCC in milk goats.[47] Therefore, a standard tool in the diagnosis of SCM in goat could be provided by bacterial culture and MSCC.

Correlates CMT score with MSCC level

The CMT is based on disrupting the membranes of somatic cells and the reaction of their DNA with an ionic surfactant. This reaction leads to changes in the milk viscosity so the higher cell content- the higher the viscosity change. This is how the CMT gives an indication of the somatic cells in the milk sample.[17]

The milk of dairy goats usually contains higher SCC compare to dairy cows and the interpretation of CMT results in goats is more complicated than in cows. The more complicated situation arises in trying to interpret SCM (trace or 1+) reactions and therefore the CMT may be best used to evaluate trends in goats to compare the results for one half of the udder with those for the other half. The presence of a clear difference in the test results between halves of the udder would provide good support for an increased SCC in one side that may require further test for milk culture and direct SCC.[48] Although the sensitivity and specificity of the CMT has been reported to be increased for the diagnosis of SCM cases,[96] it cannot be recommended to use as a single diagnostic test for diagnosis of SCM but is very useful in a screening test and selection of the animal for bacteriological examination.[13]

The indirect measurement of SCC by CMT has been reported to be well correlated with SCC measured by Portable deLaval cell counter (DCC) in dairy goats but concluded that CMT can be used as a predictor of the SCC.[54] The higher SCC have been reported in samples negative to the bacterial culture and accordingly has been suggested that the CMT can be used as a screening test in the diagnosis of mastitis in small ruminants.[97]

Prevalence of SCM associated with risk factors

Low-input smallholder goat farming systems are distinct in terms of management, productivity and hygiene status of the animals in Bangladesh. Risk factors like previous mastitis history, increased parity, poor body conditions, increased milk production, late lactation stage, long teat, housed goats and wet season have been reported to be associated with the occurrence of mastitis in goats in Ethiopia.[98] Rainy season, free-ranging farming system, poor body condition score and non-native goat breeds have been reported to be associated with significant risk factors in Chittagong, Bangladesh.[8] Some risk factors associated with SCM in lactating goats especially udder and teat related risk factors have been evaluated in this study.

Udder half-wise prevalence

This study did not find any difference on the prevalence of SCM between right and left half of the udder of lactating goats by using both the indirect and direct MSCC tests. Out of 140 udder halves screened for SCM, of which an overall 31.43% udder halves were found positive with SCM. These findings support the results of no significant difference of the prevalence of SCM between left and right half has been reported in lactating goats from Bangladesh[30] and India.[99]

The prevalence of SCM has been reported in 38.75% halves of lactating does, of which 19.17% in the left and 19.58% in the right halves with an overall animal-wise 21.67% by using CFT in Bangladesh.[30] However, significantly higher prevalence of CM has been reported in left half (79.66%) in comparison to right half (20.34%) in lactating goats of Bangladesh.[29]

Stage of lactation

This study recorded highest prevalence of SCM during late lactation (37.90%) in comparison to mid (28.10%) and early (11.10%) lactation in lactating goats. These findings are in support with the highest prevalence of SCM at late lactation during 3-4 months (100%) in comparison to mid (1-3 months) lactation (23.68- 33.33%) and 0% at early (up to 1 month) lactation.[21] However, these findings contradict with the results of highest prevalence of SCM in early (46.41%) comparison to mid (28.00%) and late (12.82%) lactations[20] and also (45.07%) in early lactation[30] in lactating goats in Bangladesh and also elsewhere.[100] These variations might be due to age, parity, breeds and management practices of the dairy goats at the different farms and rural smallholder farms.

Udder and teat factors

No significant differences was found on the prevalence of SCM in lactating goats based on udder size (diameter at the middle) between 10 to11 cm (29.40%) and >11 to 12 cm (30.60%). The highest prevalence of SCM was recorded with highest teat length of 6.0 cm (44.40%) in comparison to 5.0 cm (28.10%) and 4.0 cm (27.60%) in lactating goats. Similarly, significantly highest prevalence of SCM was recorded in lactating goats with shortest distance (9.0 cm) between teat end to floor (33.30%) in comparison with 10 cm (14.60%), 11 cm (16.70%) and 12 cm (0%). These findings support the significantly higher prevalence SCM in lactating goats has been reported with long teat.[98]

No significant difference was recorded on the prevalence of SCM in lactating goats based on teat diameter between 1.0 cm (31.30% and 2.0 cm (29.60%), whereas higher prevalence of SCM was found in lactating goats with pointed shape of teat tips (50.0%) in comparison to rounded (25.90%) teat tips. Some positive correlation between teat length and teat diameter with the prevalence of SCM in cows has been reported elsewhere.[101]

Of the four lactating goats with supernumerary teat of which two (50.0%) were affected with SCM. Out of 70 experimental lactating goats, 66 had scabies on their teats of which 28.80% affected with SCM. These observations of higher prevalence of SCM in lactating goats with teat lesions are in support with the higher prevalence of SCM with the presence of teat lesions (84.62%) than no teat lesions (14.63%) of goats[20] and (47.39%) with multiple teat lesions[30] in Bangladesh and also elsewhere.[6,102] Injury to the teats and udder facilitate access of microorganisms into the glands leading to mastitis.[103]

In addition to these recorded risk factors, several other risk factors have been incriminated to be associated with the occurrence of SCM in goats. The highest prevalence of SCM have been reported at 4 to 5 years (57.69%) of age group, 5th parity (60.61%), does having 3 kids (64.52) from Bangladesh.[30] The higher prevalence of SCM has also been reported in multiparous than primiparous goats[6] and increased of parity.[21,104] The higher age group (≥ 3 years) is epidemiologically associated with increased prevalence of SCM in goats.[53,105] More recently,

50.9% udder half level prevalence of SCM has been reported in household goats with high prevalence in late lactation, Jamnapari breed and goats with bottle shaped teats as significant risk factors for SCM in Bangladesh.[23] The increased prevalence of SCM in higher parity and aged does might be due to increased length of exposure to pathogens compared to younger does. In addition, higher parity and aged does are usually under stress resulting from long time milk production and multiple parturitions.[53]

CONCLUSIONS

Mastitis is an endemic complex multifactorial disease that is considered to be one of the most frequent and costly diseases in dairy animals worldwide including Bangladesh. This study shows the considerable high prevalence of the SCM in BBG which may have a negative impact on the health and production of goat especially poor quantity and quality of milk. This determines the importance of an early and accurate diagnosis of different forms of mastitis especially SCM in lactating goats. The use of indirect and direct SCC in milk samples for the diagnosis of SCM is suitable for application in practice and positive milk samples can be selected for further bacteriological examination for confirmatory diagnosis. However, the direct SCC technique may not be useful a single diagnostic test for SCM in lactating goats due to some biological factors. Under these circumstances, the CMT an indirect SCC method can be used in initial diagnostic test followed by culture for bacterial identification of pathogens in milk samples. A threshold of SCC of milk of 268000 / ml (CMT score 1+) has been used to differentiate between normal udder and udder with SCM. A SSC < 1,000 means the goats' udder are healthy, 2,000 to 500,000 indicates an infection by week pathogens, over a million SCC is considered a problem and over 1,500,000 SCC definitely have an infection. Certain risk factors that are associated with higher prevalence of SCM have been identified which need to be corrected for the prevention of mastitis in goats. Therefore, there is a need to improve management practices in both the organized goat farms and smallholder farms to decrease the high prevalence of SCM to a possible lower limit in Bangladesh.

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ETHICAL APPROVAL

The approval from the Institutional Animal Ethics Committee to carry out the current study was not required as no invasion procedure on the animals was performed.

CONFLICTING INTEREST

The authors declare that there is no conflict of interest to publish this article in journal.

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