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Prevalence and Associated Risk Factors of Sub-Clinical Mastitis in Lactating Cows in Selected Area of Bangladesh

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ABSTRACT

A cross-sectional study was carried out to determine the prevalence of subclinical mastitis (SCM) in the dairy farm at Shahjadpur Upazila in Sirajganj District of Bangladesh from December 2022 to December 2023. A total of 150 lactating cows was randomly selected and screened for SCM by using the California Mastitis Test (CMT). The overall prevalence of SCM at the farm level was 67.5% whereas at individual-level was 55.33% followed by quarter level (28.50%). The prevalence of subclinical mastitis was significantly higher in cross-bred cows (59.28%) compared to indigenous (40%). The cows with a daily milk yield of ≥16 L showed a higher prevalence of subclinical mastitis (69.41%). The prevalence during early lactation (£2 months) was recorded comparatively higher (59.26%). Age-wise prevalence was more prominent in ≥7-year-old cows, which was 72.09%. The prevalence of subclinical mastitis was comparatively higher (58.88%) in lactating cows that were housed with semi-paka floors compared to the muddy floor (41.18%). This study revealed the increasing prevalence with the increasing number of parities that was higher (70.00%) at ≥4th parity and lowest at 1st parity (45.00%). Descriptive statistics represented the farm demography and management status in the study area. The findings of the study may develop awareness among farmers and other stakeholders to prevent subclinical mastitis in dairy cows.

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INTRODUCTION

Livestock is a role model for development of agricultural based country like Bangladesh. The livestock sub-sector provides direct employment for 20% of the total population and partly employment for another 50% (Rahman *et al.*, 2014). Dairy farming in Bangladesh getting popular day by day and presently cattle population is 24.23 million in which around 9 million dairy cattle of crossbred high yielding cows are distributed in both household and commercial farms across the country which produces around 12 million metric ton liters of milk per year (DLS, 2021). Around 70% of farmers of Bangladesh are smallholders having 1-3 cows per farm and contribute to 70-80% of the total milk demand of the country (Uddin *et al.,* 2012; Hemme *et al*. 2008). Different type of disease problems frequently occur in dairy animals which hamper their milk production (Shamsuddoha and Edwards, 2000). Among these, mastitis is one of the most significant diseases that impede the growth of the dairy sector (Rahman *et al.,* 2014). Mastitis is a very common disease in dairy cows, associated with physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008; Islam *et al.*, 2011). The commonest pathogens in large animals are *Staphylococcus aureus, Streptococcus agalactiae*, other *Streptococcus* species and Coliforms (Sumathi et al., 2008). It causes a negative economic impact on dairy farms due to abnormal milk, deterioration of milk quality, reduced production (up to 70%), milk discharge after treatment (9%), treatment costs (7%), labor, premature culling (14%) and death (Halasa *et al.*, 2007; Kee, 2012; Bari *et al.*, 2014). The mastitis should be classified as clinical and sub-clinical form (Mpatswenumugabo *et al.*, 2017). Sub-clinical mastitis is more important and causes much higher loss to the dairy industry (Abrahmsen *et al.*, 2014). In sub-clinical mastitis, there are any clinical signs such as no abnormal milk, udder swelling or tenderness or systemic signs such as fever, depression. Instead of there is an increase in somatic cell counts of the milk (Radostits *et al.*, 2007; Kayesh *et al.*, 2014). If an early diagnosis of SCM is possible, it reduces the production loss of mastitis. For diagnosis of SCM, some techniques should be used such as California Mastitis Test (CMT), Surf Field Mastitis Test (SFMT), Somatic Cell Count (SCC), White Slide Test (WST) (Kathiriya *et al.*, 2014).

Some reports on subclinical mastitis in dairy cows has been published in Bangladesh. Tripura *et al.*, (2014) observed the overall prevalence of SCM in lactating cows is 51.8%, of which 51.4% at Mymensingh Sadar and 52.2% at Lakshmipur Sadar Upazilas in Bangladesh. A comprehensive report on sub-clinical mastitis in some area of Bangladesh are lacking till now. Some predisposing factor is responsible for SCM such as unhygienic environment, abnormally large udder, teat injury, udder wound unclean milker's hand and mismanagement of milking machine (Bari *et al*., 2014; Kathiriya *et al*., 2014). Sirajganj district is the biggest milk pocket zone in Bangladesh. The dairy sector is increasing as cultivable land decreasing; hence mastitis has a direct impact on the livelihood of the dairy farmers. So, it is necessary to know the prevalence and associated risk factors of subclinical mastitis in dairy cows to prevent mastitis and reduce losses of productivity as well. The objectives of this study were to determine the prevalence of sub-clinical mastitis in lactating cows at Shahjadpur Upazila in Sirajganj district, to know the demography of farm and farm management to increase the public awareness regarding sub-clinical mastitis.

MATERIALS AND METHODS

Study area and animals

The study was conducted in registered dairy farm at Shahjadpur Upazila in Sirajganj District of Bangladesh during December 2022 to December 2023. The geographical location of Shahjadpur Upazila is 24º10.2' North latitude and 89º35.3' East longitude with an area of 324.47 km2 (Figure 1). Most of the cows found in the study area are crossbreed. Local breed was very rare. However, the surveyed animals were the genotype of Indigenous, Local x Friesian (LxF), Friesian x Sahiwal (FxSL) Friesian x Jersey (FxJ). Animal's breed was determined on eye estimation.

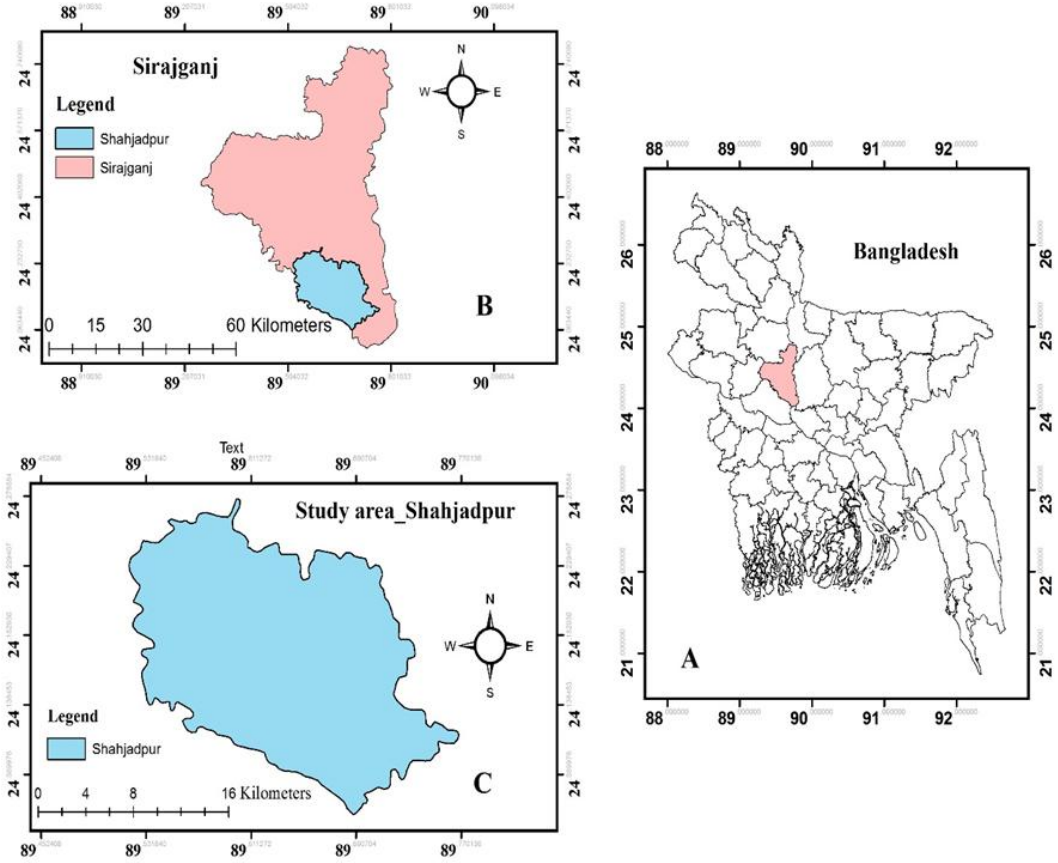

Figure 1. Geographical location of Shahjadpur Upazilla, Sirajganj

Sample size and sample collection

A total number of 40 dairy cattle farms were selected based on Union of the Upazila which covering different cattle rearing region of the study area. These dairy farms were conducted for sampling under cross sectional study design. Samples were collected from 150 clinically healthy lactating cows and the total sample size at quarter level, individual level and farm level were 600, 150 and 40 respectively. Clinically healthy was defined as normal feeding behavior and body temperature and no visible changes in udder or milk. Before collection of milk, the teat and tips were washed with clean water, antisepsis was done with a swab soaked with 70% alcohol and then milk samples were collected aseptically from the udder during morning. All the milk samples were collected in vials which were labeled with identification number of cows.

Data collection

A pre-structured questionnaire was made to take out information about farmers, farm demography, farm characteristics and farm management in form of direct interview and observations. Udder and teat shape were recorded by physical examination of cows during sample collection. The udder and teat shape were classified according to Bhutto *et al.* (2010). Finally, individual data from a SCM positive cow were gathered to determine further association of various risk factors.

California Mastitis Test (CMT)

The procedure of CMT was followed in this study as per manufacturer's instruction (Leukocyst®, Synbiotics Corporation, France). In brief, about 2 ml milk was drawn from vial into the cup and an estimated equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. Consistency normal or Gray color was indicative of negative reaction and light gel formation or purplish gray color was indicative of positive reaction (Figure 2). The CMT results were read immediately as per manufacturer's recommendation and were scored based on the amount and thickness of gel formed as described by Islam *et al.* (2011) as shown in Table 1.

Negative samples Positive samples

Figure 2. Milk Sample Evaluation by Using CMT Solution

Table 1. Scoring of California mastitis test results

Reading			**Interpretation**	
Aspect	**Score**		**Infection**	**Related with the average cellular numeration (x 10^3/ml)**
	Value	**Cross**		
Consistency normal or Gray color	0	(0)	Absent	100
Light gel disappearing after stirring or Purplish gray color	1	(±)	Infection risk by minor pathogenic	300
Light persistent gel-crumbly filaments or Purple gray	2	(+)	SCM	900
Immediate thickening viscous cluster at the bottom of the well	3	(++)	SCM	2700
Thick gel consistency of egg white color dark purple	4	(+++)	SCM near the clinical expression	8100

*SCM: Sub-clinical mastitis

Statistical analysis

Data were cleaned and entered into Microsoft Excel spreadsheet 2013 then transferred to STATA/MP 14.0 (Stata Corp LP, Texas, USA, 2014). Different variables were filed to facilitate analyses as categorical variables. Descriptive statistics were received on the data of farmer's demography, farm features, Characteristics of sampled cows and management feature of farms. The prevalence of SCM was calculated at farm level as well as individual animal level and quarter level and 95% Confidence Interval was estimated treating normal approximation.

RESULTS

Farm demography

The farmers mostly were engaged with only farming [(65%) (95% CI: 48.32-79.37)] and rest were with farming and side business from a total of 40 dairy farms in the study area. The farm size was medium scale ranged from 6-15 cows (52.50%) followed by small (<6) and large (>15) scale with financed mainly by NGO (27.5%). They principally reared their cows in intensive housing system (55%) which are made up of semi-paka floor (57.50%). Hossain *et al.* (2004) reported that 63% were reared by intensive system and 34% were reared semi intensive system which is almost supported the present study (Table 2). The farm had semi paka (brick) floor (57.50%) and others had concrete (27.50%) and muddy (15.00%) floor which was supported by Rahman et al. (2009).

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Table 2. Descriptive analysis of farm demography at farm level in the study area (N=40)

Variable	Category	Frequency (%)	95% Confidence Interval
Occupation	Farming	26 (65.00)	48.32-79.37
	Farming and Business	14 (35.00)	20.63-51.68
Source of Finance	Own	13 (32.50)	18.57-49.13
	Bank	10 (25.00)	12.69-41.20
	NGO	11 (27.5)	14.60-43.89
	Relatives	6 (15.00)	5.71-29.84
Farm Type	Small (<6)	13 (32.5)	18.57-49.13
	Medium (6-15)	21 (52.50)	36.13-68.49
	Large (>15)	06 (15.00)	5.71-29.84
Hygiene Condition	Poor	20 (50.00)	33.80-66.20
	Moderate	20 (50.00)	33.80-66.20
Rearing System	Intensive	22 (55.00)	38.49-70.74
	Semi-Intensive	18 (45.00)	29.26-61.51
Floor-type	Muddy	6 (15.00)	9.05-35.65
	Semi-paka	23 (57.50)	40.89-72.96
	Concrete	11 (27.50)	14.60-43.89
Bedding material	Rubber mat	11 (27.50)	14.60-43.89
	Absent	29 (72.5)	56.11-85.39
Semen Source	Milk Vita	17 (42.50)	27.04-59.11
	Brac	14 (35.00)	20.63-51.68
	Government	9 (22.5)	10.84-38.45

Farm management

In this study, it was found that among the cows (N=150) Holstein Friesian Crossbred cows were the predominant (N=101, 67.3%) whereas local indigenous, Sahiwal cross and Jersy cross were 6.67%, 14.67% and 11.33% respectively. The investigated cows mostly belonged 5-7 years of age (42%) and then 2-4 years (29.33%) and above 7 years (28.67%). Of the lactating animals, 44.67% were in ≥7 months of lactation period, correspondingly 37.33% were in 3-6 months and 18% were in ≤2 months. We observed milk production status of the sampled cows and it was noticed that 30% of the cows produced ≥16 liters of milk per day followed by 6-15 liters (56.67%) and ≤5 liters (13.33%) (Table 3).

Table 3. Descriptive analysis of dairy animal's demography in the study area (N=150)

Variable	Category	Frequency %)	95% Confidence Interval
Genotype	Indigenous	10 (6.67)	3.24-11.92
	Holstein Friesian Cross	101 (67.3)	59.21-74.76
	Sahiwal Cross	22 (14.67)	9.43-21.36
	Jersy Cross	17 (11.33)	6.74-17.52
Age (years)	2-4	44 (29.33)	22.19-37.31
	5-7	63 (42.00)	34.00-50.32
	>7	43 (28.67)	21.59-36.61
Body Weight (kg)	150-250	56 (37.33)	29.58-45.60
	251-350	77 (51.33)	43.05-59.57
	>350	17 (11.33)	6.74-17.52
BCS	2	50 (33.33)	25.86-41.48
	≥3	100 (66.67)	58.52-74.14
Parity	1st	40 (26.67)	19.78-34.49
	2nd	23 (15.33)	9.98-22.11
	3rd	28 (18.67)	12.78-25.84
	4th	29 (19.33)	13.35-26.57
	>4th	30 (20.00)	13.92-27.30
Pregnancy	Pregnant	70 (46.67)	38.49-54.98
	Non-pregnant	80 (59.33)	45.02-61.51
Lactation Period (Month)	≤2	27 (18.00)	12.21-25.10
	3-6	56 (37.33)	29.58-45.60
	≥7	67 (44.67)	36.55-52.99
Udder Shape	Pendulous	90 (60.00)	51.69-67.90
	Others	60 (40.00)	32.10-48.31
Teat Shape	Round	32 (21.33)	15.07-28.76
	Pointed	77 (51.33)	43.05-59.57
	Others	41 (27.33)	20.38-35.20
Types of stimulation used during milking	Calf suckling	121 (80.67)	73.43-86.65
	Oil	29 (19.33)	13.35-26.57
Milk Production (Liter)	≤5	20 (13.33)	8.34-19.84
	6-15	85 (56.67)	48.34-64.73
	≥16	45 (30.00)	22.80-38.01
Previous History of mastitis	Yes	70 (46.67)	38.49-54.98
	No	80 (53.33)	45.02-61.51

Prevalence of Sub-clinical mastitis in the study area

The overall prevalence of SCM at the farm-level was 67.5% whereas at individual cow level was 55.33% and at quarter level was 28.50% (Table 4). This finding is in line with the studies of Biswas *et al.*, (2017) in Barishal who reported 51.56%. Badiuzzaman *et al.* (2015) revealed that the prevalence of SCM was 61.26% in Rajshahi. Mpatswenumugabo *et al*. (2017) reported that the prevalence of SCM was 88.6% in Vietnam. Kayesh *et al*. (2014) studied that the prevalence of SCM at quarter level was 26.13% in Barisal. The difference in prevalence rates of SCM might be due to geographical location, management system and test used for screening of milk samples (Barua *et al*., 2014).

Table 4. Overall Prevalence of Sub-Clinical Mastitis in the study area

Variable	N	Frequency	P (%)	95% CI
Quarter	600	171	28.50	24.92-32.30
Individual	150	83	55.33	47.01-63.45
Farm	40	27	67.5	50.87-81.43

Prevalence of Sub-clinical mastitis based on various factors

This study revealed on the basis of breed that crossbred cows showed high prevalence in comparison to indigenous cow (40%). On the other hand, HF crossbred cows showed 60.39% (n=61 of 101) positive reaction to CMT among the crossbred cows. The prevalence of SCM in the study area at >7 years old cows was higher (72.09%) in comparison to 5-7 years (46.03%) and 2-4 years (57.27%) (Table 5). Uddin *et al.* (2010) and Rahman *et al.* (2009) reported 77% of prevalence at 7-8 years of age and 42.5% at 5-6 years of age respectively which are inclined with this study.

The overall prevalence of sub-clinical mastitis based on lactation stage in cows showed that all the three lactation stages had sub-clinical mastitis but, the highest prevalence of sub-clinical mastitis was recorded in ≤2 months of lactation (59.26%, 95% CI: 43.22-70.29) which was followed by 3-6 months (55.36%, 95% CI: 44.04-68.78) and ≥7 months (47.76, 95% CI: 28.67-68.05). Hasan *et al.* (2022) reported with the relation of this study, the prevalence of SCM at ≥1 month of lactation was 43.20%. On the other hand, Badiuzzaman *et al.* (2015) revealed 78.43% at ≥3 months of lactation.

The results of prevalence of sub-clinical mastitis based on the parity number of cows are presented in Table 5. Based on parity number of cows, the highest prevalence was 70.00%, in ≥4th parity of cows, whereas in 1st and 2nd parity the prevalence was 45.00% (95% CI: 29.26-61.51) and 52.17% (95% CI: 30.59-73.18), respectively. Uddin *et al.* (2010) reported similar findings which was 73.47% at 3rd parity. Based on daily milk yield, the prevalence of sub-clinical mastitis recorded was 50% (95% CI: 40.66-62), 55.56% (95% CI: 40.00-70.35) and 69.41% (95% CI: 45.72-88.11) in lactating cows with daily milk yield of ≤5 L, 6-15 L, and ≥15 L, respectively. This finding is in line with the finding of Rahman *et al.* (2010) who reported 63.30% of prevalence in lactating cows having 16-20 L of milk.

Table 5. The Prevalence of sub-clinical mastitis based on various factors (N=150)

Factors	Category	No. of cows (N)	Frequency (%)	95% Confidence Interval
Genotype	Indigenous	10	4 (40.00)	18.44-67.08
	HF Cross	101	61 (60.39)	26.24-87.84
	Sahiwal Cross	22	13 (59.09)	36.35-79.29
	Jersy Cross	17	9 (52.94)	46.20-66.28
Age (years)	2-4	44	23 (57.27)	36.69-67.54
	5-7	63	29 (46.03)	33.39-59.06
	>7	43	31 (72.09)	56.33-84.67
Floor-type	Muddy	17	7 (41.18)	18.44-67.08
	Semi-paka	107	62 (58.88)	48.95-68.30
	Concrete	26	14 (53.85)	33.37-73.41
Parity	1st	40	18 (45.00)	29.26-61.51
	2nd	23	12 (52.17)	30.59-73.18
	3rd	28	16 (57.14)	37.18-75.54
	4th	29	16 (55.17)	35.70-73.55
	>4th	30	21 (70.00)	50.60-85.27
Pregnancy	Pregnant	70	40 (57.14)	44.75-68.91
	Non-pregnant	80	43 (53.75)	42.24-64.97
Lactation Period (Month)	≤2	27	16 (59.26)	43.22-70.29
	3-6	56	31 (55.36)	44.04-68.78
	≥7	67	32 (47.76)	28.67-68.05
Milk Production (Liter)	≤5	20	10 (50.00)	40.66-62
	6-15	45	25 (55.56)	40.00-70.35
	≥16	85	59 (69.41)	45.72-88.11
Previous History of mastitis	Yes	70	51 (72.85)	60.90-82.80
	No	80	32 (40.00)	29.20-51.56

The prevalence of sub-clinical mastitis on reproductive state was presented in the table 5. The prevalence of sub-clinical mastitis was higher in pregnant cows 57.14% (95% CI: 44.75-68.91) compared with non-regnant cows 53.75% (95% CI: 42.24-64.97). Based on previous history of mastitis, the highest prevalence of sub-clinical mastitis in previously affected cows 72.85% (95% CI: 60.90-82.80) and respectively 40.00% (95% CI: 29.20-51.56) in previously not affected in mastitis.

CONCLUSION

The results of prevalence and identified associated risk factors of Sub-clinical mastitis unlock that SCM is the major threat for continuing development of dairy industry in Bangladesh. California Mastitis test kit (CMT) is the conventional, gold standard screening test kit for SCM. Early detection of SCM in dairy farms and focusing on identified risk factors can be the ideal pathway to prevent clinical mastitis in cow.

CONFLICT OF INTEREST

There is no conflict of interest

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