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Article

Prevalence of sub-clinical mastitis and associated risk factors at cow level in dairy farms in Southwestern part of Bangladesh

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Abstract: A cross-sectional study was carried out to determine the prevalence of subclinical mastitis (SCM) among medium to large scale household dairy farms in southwestern district, Jhenaidah, Bangladesh during July to December 2019. A total of 78 (n=100) lactating cows from household dairy farms (N=32) having three or more dairy cows were selected randomly as sampled populations. Milk samples were screened for SCM by using Surf Field Mastitis Test (SFMT). The prevalence of SCM varied among farm level [71.9% (95% CI: 53.3-86.3)], individual animal level [67.9% (95% CI: 56.4-86.3)] and quarter level [29.5% (95% CI: 24.5-34.9)]. Descriptive statistics represented the farmers and farm demography, characteristics of the sampled population, and overall management feature. Random Effect Logistic Regression identified, Body Condition Score (BCS) [OR=3.8 and 2.9, at cows level and quarter level respectively (BCS-2 vs. BCS-≥3)], and breed [OR=5.1 and 2.9, at cows level and quarter level respectively (HF× Sahiwal vs. HF × Local)] as potential risk factors. This study shows that SCM is highly prevalent in the study area, which is a major threat to the dairy industry's production performance. Regular screening by SFMT, proper hygiene, improve the management system, and farmer's awareness is required to control the disease.

Keywords: Sub Clinical Mastitis, prevalence, Surf Field Mastitis Test, Jhenaidah

1. Introduction

Dairy farming in Bangladesh getting popularity day by day and presently around 6 million dairy cattle of crossbred high yielding cows are distributed in both household and commercial farms across the country which produces around 9.4 million metric ton liters of milk per year [1, 2]. Production disease like mastitis is the major hindrance of getting the optimum benefit from a dairy farm [3]. In Bangladesh, mastitis impedes the dairy sector's growth due to decreased production [4]. Mastitis generates a considerable loss to the dairy industry, which has been estimated for Bangladesh as Tk. 122.6 (US $2.11) million per year [5].

Clinical mastitis causes a negative economic impact on dairy farms through 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 [5-7], whereas subclinical mastitis (SCM) is in the absence of clinical signs rather than an increase in somatic cell counts of the milk [8, 9]. Subclinical mastitis is 15 to 40 times more prevalent than that of clinical mastitis, is of long duration

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[10-12]. Many earlier studies have been reported the SCM status in Bangladesh along with neighboring countries, including India, Sri Lanka, and Pakistan as well[12-15]. In Bangladesh, the prevalence of SCM in the crossbred dairy cows has been documented as 28.5-61.3%[8, 15-17]. Unfortunately, no study has been found on SCM in the southwestern region, especially at the Jhenaidah district of Bangladesh.

Several techniques including California Mastitis Test (CMT), Surf Field Mastitis Test (SFMT), Somatic cell Count (SCC), White Slide Test (WST) are used in field condition for SCM diagnosis [12] among which SFMT has possessed a sensitivity of 72.8% and specificity 87.1% and available in field condition at a very reasonable cost [15, 18]. So, this study has used SFMT technique to popularize it in field conditions.

Multiple studies have been carried out to identify the risk factors of SCM in lactating cows in different countries, including Bangladesh [11, 14]. The most-reported predisposing factor for SCM at farm level was the unhygienic environment, abnormally large udder, teat injury, udder wound, unclean milker's hand, and mismanagement of milking machine [5, 12]. Some other risk factors that can hardly be influenced through management are age, parity, stage of lactation and housing; whereas breeding, teat shape and body condition score (BCS) are included as manageable risk factors [5, 19, 20]. Again, teat end to floor distance, pregnancy, milk yield, ways of milking stimulation with milking technique, milk leakage, and type of floor have also been reported as the most significant risk factors of mastitis by different authors [14, 16]. The knowledge of SCM risk factors is needed to provide farmers with advice to prevent clinical mastitis in their cows. Therefore, the present study has been conducted to estimate the prevalence and risk factors associated with sub-clinical mastitis (SCM) in Jhenaidah district

2. Materials and Methods

2.1 Study area

The study was conducted in the registered household dairy farms in three selected Upazilas (Jhenaidah Sadar, Harinakunda and Kotchadpur) of Jhenaidah district of Bangladesh during the six months from July to December 2019. The study area is geographically located at 23.54^0N and 89.00^0E at the southwestern region of Bangladesh, surrounded by Jessore district at the south, Kushtia at the north, Rajbari,and Magura at east, and Chuadanga at western border however Indian border at the southwestern part of this area [21]. The district comprises 6 Upazila, located geographically at a tropical climatic area with an annual temperature ranging from 11.2 to 37.10^0 C, and average rainfall is1467mm[22] (Figure 1).

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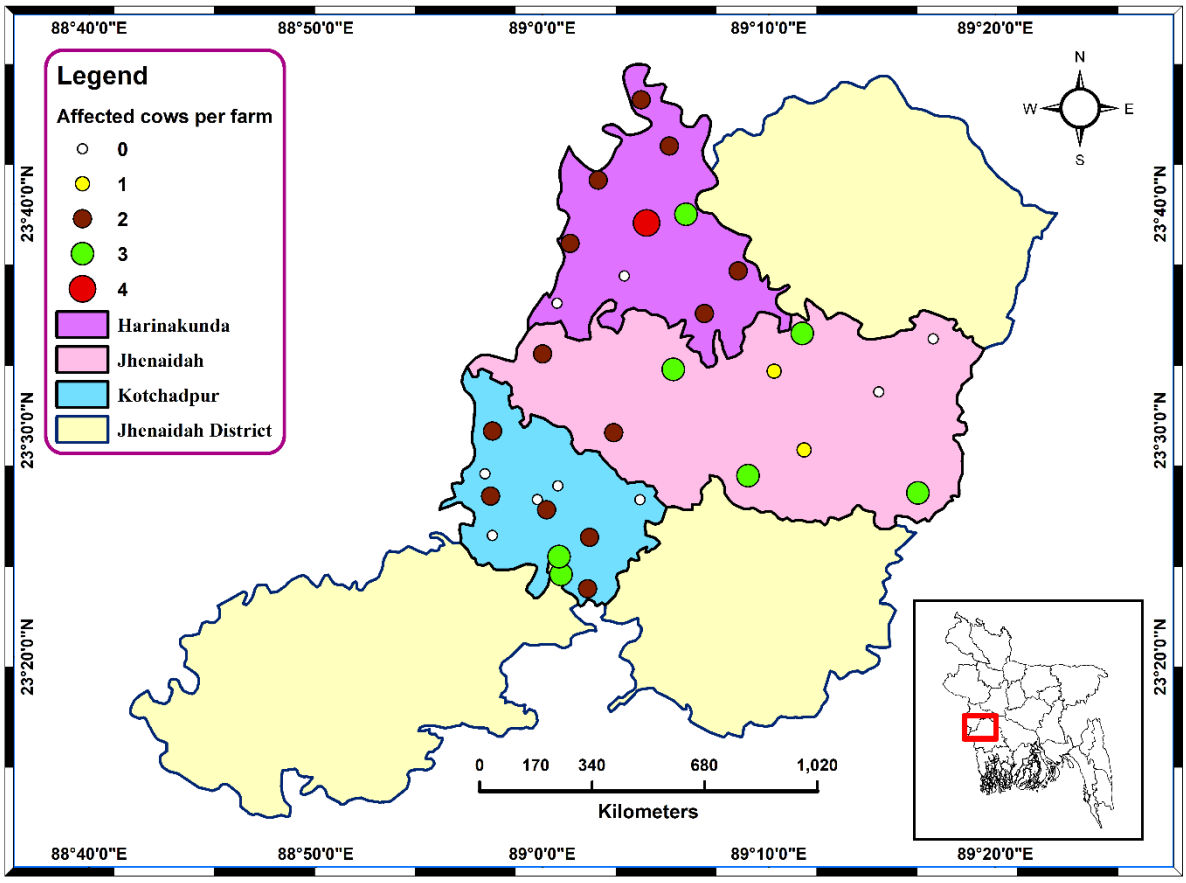

Figure 1: Spatial location of the study site with several positive samples per farm (some symbol of farm location is overlapped due to proximity with another farm)

2.2 Reference population, Sample size, and sampling

Among six Upazila of Jhenaidah district, we selected three Upazila (Jhenaidah Sadar, Harinakunda and Kotchadpur) based on easy accessibility to the field to conduct the study. All medium-to large sized commercial household dairy farms recorded at District Livestock Office having at least one dairy lactating cow per farm during the study period in the study area were considered as the reference population. Thus, a complete list of household dairy farms (N=32 with 100 lactating cows) was prepared using the data obtained from the District Livestock Office of Jhenaidah.

To investigate the prevalence of SCM at the animal level, a total of 78 (n=100) individual lactating cows were required to evaluate considering 50% expected SCM prevalence (as no previous study has been reported SCM prevalence in this study area), ±5% precision, with a 95% confidence interval (https://www.openepi.com/SampleSize). The samples were collected from all the clinically healthy lactating cows under 32 commercial household dairy farms. Clinically healthy cows were defined as normal feeding behavior and body temperature and no visible changes in udder or milk.

2.3 Reagent preparation and SFMT sensitivity analysis

SCM reagent was prepared as the recipe described by Muhammad, Naureen [18] and Bachaya, Raza [23]. A, 3% surf solution (P^H=10.3) was briefly prepared by adding 3 gm detergent powder (Surf Excel®, Unilever, Bangladesh Ltd.) in 100ml of distilled water.

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2.4 Milk sample evaluation for SCM

In the current study, milk samples were evaluated based on the method described by Bachaya, Raza [23] in brief, after discarding 1-2 squirts of foremilk, 2ml milk and 2ml of SFMT solution were mixed to a CMT paddle, and it was twirled up to 20 to 30 seconds and observe the appearance of gel which was graded from 1 to 5 and interpreted as described by Iqbal, Amjed [24] and Sharif, Ahmad [25]. Normal consistency of milk was scored +1, indicating Negative(Fig. 2/A), whereas Light gel disappearing after stirring was scored +2, showing Trace positive (Fig. 2/B). Again, light persistent gel short filaments were scored +3, indicating first degree positive for SCM (Fig. 2/C), Immediate thickening viscous cluster at the bottom of the well was scored +4, indicating second degree positive for SCM (Fig. 2/D). However, thick gel consistency of egg albumin was scored +5, indicating third-degree positive SCM near clinical expression. No cows were found third-degree positive with a score of +5 in this study. The test score, 2 or more, was considered as SCM positive. If a sample from any quarter of four scored 2 or more, then that animal was treated as positive for SCM, and if any lactating cattle scored 2 or more, then the farm was defined as positive to SCM.

Figure 2: Milk sample evaluation using SFMT solution. A) Normal consistency of milk indicating negative, B) Light gel disappearing after stirring showing trace positive, C) Light persistent gel short filaments indicating first degree positive, D) Immediate thickening viscous cluster at the bottom of the well indicating second degree positive

2.5 Data collection

A pre-structured questionnaire was developed and reviewed under Udder Health Development Project (https://uhb.org.bd) of Bangladesh run by Chattogram Veterinary and Animal Sciences University in collaboration with Utrecht University, The Netherlands and Swedish National

Veterinary Institute, Sweden. The questionnaire was structured on farmers and farm demography, farm characteristics, and farm management. The data were recorded in the paper-based questionnaire by face to face interview and direct observation. The physical examination of cows recorded udder and teat shapes during sample collection. The udder and teat shapes were classified according to Bhutto, Murray [26]. One hour was taken for each interview to fill the questionnaire. The cross-breed composition was recorded based on the history of artificial insemination in the previous parent stock of the sampled animals.

2.6 Statistical analysis

Sample evaluation and field data were cleaned and entered into Microsoft Excel spreadsheet 2007, and then transferred to STATA/MP 14.0 (Stata Corp LP, Texas, USA). Different variables were filed to facilitate analyses as categorical variables where quantitative variables were categorized based on percentile. Body Condition Score (BCS) was evaluated, as described by Roche, Friggens [27]. Descriptive statistics were received on the data of farmer's demography, farm features, characteristics of sampled cows, and management features of farms. The prevalence of SCM was calculated at the farm level as well as the individual animal level and quarter level, and 95% Confidence Interval was estimated. The descriptive outputs were expressed in frequency value, percentage, and 95% CI.

A univariate chi-square test was performed to investigate the relationship between different variables and SCM. The variable indicating significant in univariate analysis ($P \leq 0.05$) was forwarded for multivariate analysis as described by Sayeed, Smallwood [28]. The difference between likelihood-based standard error and robust (residual-based) standard error of logistic regression for both individual animal level and quarter level data indicate statistical clustering where particular farm and the individual animal was a cluster, respectively. A random-effect model was fitted up using a backward elimination process for both individual and quarter-level analyses and checking for collinearity using chi-square statistics between independent variables. Hence two factors- frequency of milking (once and >once in a day) and previous history of mastitis (yes/no) was significant in univariate analysis. Due to collinearity with both Body Condition Score (BCS) and Crossbreed, those two factors were dropped from multivariate analysis. The final outputs of the model were expressed as Odds Ratio (OR), wald test p-value, and 95% CI.

3. Results

3.1 Farm Demography

In Jhenaidah, 87.5% of total farmers (n=32) responded dairy farming as their main occupation. Maximum farmers were educated at a different level, where 9.4% offarmers were illiterate. Farm housees were mostly made up of concrete (65.6%) and semi-concrete (34.4%), but 59.4% had no boundary/fence in their farms. Most of the farms had concrete floor (78.1%) followed by brick (12.50%) and earthern (9.4%) floor; rubber mattress was predominantly used in the farms (75.0%). Around 78.1% of farms had a well-constructed drainage facility, and 59.4% of farmers have cleaned the floor twice daily, followed by thrice daily (21.9%) and once daily (18.8%). About 53.1% of farms had their fodder land. Only 43.8% of farmers had professional training on dairy farming organized by the Upazila livestock office. Most farmers followed the intensive rearing (75.0%), followed by semi-intensive farming (25.0%). For artificial insemination, 53.1% of farmers used the semen supplied by the government,whereas, 46.9% of farmers used the semen provided by Bangladesh Rural Advancement Committee (BRAC). Nevertheless, there had no quarantine and footbath facility on the farm (Table 1).

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Table 1: Descriptive of farm demography and basic management info at farm level in Jhenaidah district (N=32).

Variable	Category	Frequency (%)	95% Confidence Interval
Occupation	Farming	28 (87.5)	71.0-96.5
	Farming and small business	4 (12.5)	3.5-29.0
Educational status	Illiterate	3 (9.4)	2.0-25.0
	≤Class 8	20 (62.5)	43.7-78.9
	>Class 8 to SSC	6 (18.8)	7.2-36.4
	>SSC	3 (9.4)	2.0-25.0
Types of House	Concrete	21 (65.6)	46.8-81.4
	Semi concrete	11 (34.4)	18.6-53.2
Boundary	Concrete	12 (37.5)	21.1-56.3
	Iron rod	1 (3.1)	0.08-16.2
	Absent	19 (59.4)	40.6-76.3
Floor	Concrete	25 (78.1)	60.1-90.7
	Brick	4 (12.5)	3.5-28.9
	Muddy	3 (9.4)	1.9-25.1
Bedding material	Rubber mat	24 (75.0)	56.6-88.5
	Absent	8 (25.0)	11.5-43.4
Drainage facility	Present	25 (78.1)	60.1-90.7
	Absent	7 (21.9)	9.3-39.9
Frequency of cleaning floor	Once	6 (18.8)	7.2-36.4
	Twice	19 (59.4)	40.6-76.3
	Thrice	7 (21.9)	9.3-39.9
Own fodder land	Yes	17 (53.1)	34.7-70.9
	No	15 (46.9)	29.1-65.3
Professional training on farming	Yes	14 (43.8)	26.4-62.3
	No	18 (56.3)	37.7-73.6
Breed	Cross	32 (100.0)	89.1-100*
Rearing system	Intensive	24 (75.0)	56.6-88.5
	Semi intensive	8 (25.0)	11.5-43.4
Semen Source	BRAC	15 (46.9)	29.1-65.3
	Government	17 (53.1)	34.8-70.9

3.2 Farm Animal Demography

Most of the farms were based on an intensive rearing system and preferred crossbred animals such as HF crossed withlocal indigenous cattle (85.9%)followed by HF crossed withSahiwal(14.1%). Around 37.2% of farms' herd size of milch cows was between 3 to 4, 35.9% of farms' herd size was ≤2, and 26.9% of farms' herd size was ≥5. Around 38.5% of animals were in ≤3.5 years old, whereas 38.5% and 23.1% of animals were between 3.6 and 5 and >5 years old, respectively. Again, 44.9% of animals' body weight was ≤320 kg, and 55.1% of animals' body weight was ≥321 kg. About 60.3% of animals were in BCS ≥3, whereas 39.7% was in the BCS2 category. Most of the lactating animals were in ≥3rd parity (44.9%), whereas 55.1% of animals were in ≤2nd parity. Among the lactating animals, around 67.9% were pregnant and 32.1% as non-pregnant. Almost 75.6% of farms practice milking twice or more in a day. Overall, 39.7% of animals produced ≤10 liters of milk per day, whereas 50.0% of animals produced 11-20 liter of milk, and around 10.3% of animals produced ≥21 liters of milk. About 44.9% ofsampled animals were previously affected with mastitis (Table 2).

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Table 2: Descriptive analysis of farm demography at the individual level in Jhenaidah district (N=78).

Variable	Category	Frequency (%)	95% Confidence Interval
Crossbred	HF × Local	67 (85.9)	76.2-92.7
	HF × Sahiwal	11 (14.1)	7.3-23.8
Age (years)	≤3.5	30 (38.5)	27.7-50.2
	3.6-5.5	30 (38.5)	27.7-50.2
	≥5.5	18 (23.1)	14.3-34.0
Body weight (kg)	≤320	35 (44.9)	33.6-56.6
	≥321	43 (55.1)	43.4-66.4
BCS	≥3	47 (60.3)	48.5-71.2
	2	31 (39.7)	28.8-51.5
Parity	≤2	43 (55.1)	43.4-66.4
	≥3	35 (44.9)	33.6-56.5
Herd size (No. of milch cow)	≤2	28 (35.9)	25.3-47.5
	3-4	29 (37.2)	26.5-48.9
	≥5	21 (26.9)	17.5-38.2
Dry cow	≤2	31 (56.4)	42.3-69.7
	≥3	24 (43.6)	30.3-57.7
Pregnancy	Non-pregnant	25 (32.1)	21.9-45.6
	Pregnant	53 (67.9)	56.4-78.1
Lactation stage	Early	31 (39.7)	28.8-51.5
	Mid	31 (39.7)	28.8-51.5
	Late	16 (20.5)	12.2-31.2
Lactation period (month)	≤2	31 (39.7)	28.8-51.5
	3-5	28 (35.9)	25.3-47.5
	≥6	19 (24.4)	15.3-35.4
Udder shape	Bowl	10 (12.8)	6.3-22.3
	Cup	1 (1.3)	0.03-6.9
	Tight	57 (73.1)	61.8-82.5
	pendulous	10 (12.8)	6.3-22.3
Teat shape	Cylindrical	12 (15.4)	8.2-25.3
	Flat	3 (3.9)	0.8-10.8
	Pointed	61 (78.2)	67.4-86.8
	Round	2 (2.6)	0.3-8.9
Types of stimulation used during milking	Calf/suckling	75 (96.2)	89.2-99.2
	Hand	3 (3.9)	0.8-10.8
Frequency of milking	1	19 (24.4)	15.3-35.4
	≥2	59 (75.6)	64.6-84.7
Milk production (liter)	≤10	31 (39.7)	28.8-51.5
	11-20	39 (50.0)	38.5-61.5
	≥21	8 (10.3)	4.5-19.2
Calf suckling practices after milking	Calf not suckling	5 (6.4)	2.1-14.3
	Calf suckling	73 (93.6)	85.7-97.9

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Previous history of mastitis	Yes	35 (44.9)	33.6-56.5
	No	43 (55.1)	43.4-66.4
Average daily milk yield before mastitis (liter)	≤10	6 (17.1)	6.6-33.6
	11-20	18 (51.4)	34.0-68.6
	≥21	11 (31.4)	16.9-49.2
Average daily milk yield after mastitis (kg)	≤2	7 (20.0)	8.4-36.9
	2.1-3.5	12 (34.3)	19.1-52.2
	≥4	16 (45.7)	28.8-63.3

3.3 Prevalence of Sub Clinical Mastitis in Jhenaidah district

The prevalence varied among farm level, individual animal level, and quarter level as well. The overall prevalence of SCM 71.9% at farm level, 76.9% at cow level and 29.5% at quarter level (Table 3).

Table 3: The Prevalence of subclinical mastitis in Jhenaidah district (N=32 farms, 78 cows and 312 quarters)

Level	**Frequency (%)**	**95% Confidence Interval**
Quarter	92 (29.5)	24.5-34.9
Individual	53 (67.9)	56.4-86.3
Farm	23 (71.9)	53.3-86.3

Geographically, farm level SCM was more prevalent in Jhenaidah(90.0%) followed by Kotchadpur(83.3%) and Harinakunda(60.0%). Among individual animal levels, the highest prevalence was estimated in Kotchadpur at 73.3% followed by Jhenaidah Sadar 68.0% and Harinakunda (60.9%). Again, in quarter level, the highest prevalence was estimated in Jhenaidah Sadar (42.0%) followed by Kotchadpur (25.0%) and Harinakunda (21.9%) (Table S1).

3.4 Risk Factors for SCM

The factors determined as significant in univariate analysis was nominated for multivariate analysis (Table S2). The estimated odds ratio of SCM was 3.8 times higher among individual animals having BCS 3 or more in contrast to BCS 2. In contrast, the odds ratiowas 2.9 times higher at the quarter level of the same BCS group. Again, the odds of SCM was 5.1 times higher among the individual animals of having HF and Local crossed blood than the animals of HF and Sahiwal cross blood. However, the odds of SCM was 2.9 times higher among the HF × Local rather than HF× Sahiwal at quarter level (Table 5).

Table 4: Multivariate association between individual-level and quarter level potential risk factors and SFMT score

Factors	**Category**	**Animal Level**			**Quarter Level**		
		OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Body Condition Score (BCS)	2	Ref			Ref		
	≥3	3.8	1.3-11.1	0.01	2.9	1.3-6.7	0.01
Breed	HF× Sahiwal	Ref			Ref		
	HF × Local	5.1	1.3-18.9	0.01	2.9	0.9-9.1	0.06

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4. Discussion

4.1 Farmers and farm demography

In Jhenaidah district, farmers reared crossbreed animals following a predominantly intensive system supported by the study conducted byHossain, Hossain [29]. The present study revealed that most of the dairy farm of Jhenaidah had concrete floor 78.1%, and others had brick 12.5% and muddy 9.4% floor, which is in line with the study conducted byHossain, Hossain [29] at Rangpur, Bangladesh. Most of the farmers used Rubber mat to protect the cows from the slippery condition. Again,Hossain, Hossain [29] also reported that 73.0% of cowshed had proper drainage systems, which are similar to the present study. Almost all of the farmers were preferred to artificial insemination, and most of them used from government-supplied semen, which may be due to easy availability at a very reasonable price. However,the current study revealed that 53.1% of farmers have their fodder land supported by the study [30]. Around 43.8% of dairy farmers received training at least once for dairying, which is much higher than the study conducted by Hossain, Alam [30] reported that about 17% of dairy farmers received training. This variation might be due to differences in the farmers' consciousness due to geographical location and socioeconomic status.

4.2 Prevalence of SCM

The prevalence of SCM at the farm level was 71.9%, which might be a matter ofsignificant anxiety for a dairy farmer in Jhenaidah district. However, the prevalence of SCM at the individual cow level was 67.9%, which has consistent and discrepancies with the study reporting prevalence in Bangladesh and around the globe. The estimated prevalence is higher than the reported prevalence of SCM in different district of Bangladesh including Sirajganj, Rajshahi, Chattogram and Barisal [8, 15, 17, 31]again the reported SCM prevalence is lower than the prevalence of SCM calculated in Nigeria (85.3%) [32], Uganda (86.2%) [33] and Vietnam (88.6%) [34]. However, the estimated SCM prevalence is higher than the study conducted inKenya[35], Ethiopia [36], New South Wales of Australia[37], Rwanda [19]and Uruguay [38]. The variation of SCM prevalence within the country and around the globe might be due to the geographical location, climatic condition, farm composition, and overall husbandry practice [31].

Quarter level prevalence of SCM is in close agreement with the studies conducted in different parts of the country, including Barisal [8] Mymensingh and Tangail [20] but much lower than Rajshahi [17]district of Bangladesh. Moreover, the estimated value is much higher than the reported prevalence ina neighboring country like Pakistan[14] andIndia [39]. The differences in prevalence between studies might be due to differences in milking practice, environmental conditions, and animals' immune status, which is supported by Qayyum, Khan [14].

4.3 Risk factors of SCM

The present study corroborates with the other study findings reporting risk factors of SCM in Bangladesh and other countries.The study identified a significant-close association between parity and SCM prevalence,which can be supported by [31],who reported a significant increase of mastitis among cows at ≥4th parity at quarter level (P=0.02). At the individual animal level, there was an insignificant (P=0.24) increase of SCM prevalence at ≥4th parity in contrast to ≤3rd parity. In Ethiopia,Mekibib, Furgasa [40] reported significantly (P=0.001) higher prevalence of SCM at an individual cow level of ≥4th parity. However, different studies in Srilanka [13]and Pakistan [14] revealed an increasing tendency of SCM with an increase in the number of parity.

Besides, we revealed a near to close significant association between the different udder shape and SCM prevalence at a quarter level which can be supported by the study conducted in Mymensingh and Rajshahi district of Bangladesh [11, 41]and Pakistan as well[14].

Contrarily, frequency of milking has a significant association identified in univariate analysis for both animal and quarter level which can be metaphorized byKlaas, Enevoldsen [42] who reported that increased milking frequency make the teat canal open for much time in a day which ultimately enhances the chance of udder infection. Again, the cows with previous mastitis history have been identified with significantly higher SCM prevalence in the present study that might be due to not complete healing of the udder tissue or may be due to misclassification during the study.

The present study revealed a strong significant association between the prevalence of SCM and BCS in both univariate and multivariate analyses. These findingsare in line with the study conducted by Sarker, Parvin [11]. They reported that animals with higher BCS might produce more milk that makes them prone to SCM,supported by Haile-Mariam, Bowman [43], which might be due to decrease lymphocytic functions [44]. Besides, cows with higher BCS might have more chance of suffering from metabolic diseases [45], make them more susceptible to other infectious diseases like mastitis supported by Chagunda, Larsen [46] and Moyes, Larsen [47]. However, the present study estimated a significant relation of different categories of crossbreed cows with the prevalence of SCM both in univariate and multivariate analyses. As a crossbreed of HF and Local having higher OR (5.1) in contrast to a crossbreed of HF and Sahiwal which can be supported by the study conducted in Chattogram, Bangladesh revealed a higher prevalence of SCM among the crossbreed species of HF and Local[31]. In contrast, the study conducted by Sanotharan, Pagthinathan [13] reported a comparatively lower prevalence of SCM among the Sahiwal breed in Srilanka. This statement can be concluded that Sahiwal is a temperate breed with more milk producibility and resistance to mastitis so, the cross of HF and Sahiwal are well tolerated than local, and HF cross-breed progeny [48, 49].

5. Conclusions

Subclinical mastitis is considered to be an important challenge for dairy development. The prevalence of SCM is 71.9%, 67.9%, and 29.5% calculated at farm level, individual animal level, and quarter level, respectively. The animal with BCS-≥3 and crossbreed cows of HF × Local are more likely to be infected with SCM. This output is an indication of the prevalent of SCM in the study area. To stop the progression of SCM to CM, it needs to control SCM at its early stage. So, SFMT is a cost-effective and easily applicable technique for regular screening of SCM. Proper hygiene should be maintained in every aspect of dairy farms. Care and management should be improved, and the farmers should be aware of the economic importance of the disease.

Limitations

1. Small sample size due to application of a clause “farm with at least three or more lactating cows”.
2. The unwillingness of the farmer to test their cows fearing, reducing milk demand.
3. The majority of the farm does not have a proper record book, and they were not interested in disclosing the disease information.

Supplementary Materials: Table S1, Table S2

Authors contribution: "Conceptualization, M.A.S. and M.A.R.; methodology, M.A.S. and M.A.R.; fieldwork,M.A.S. and M.A.R.; data analysis, M.A.S.;writing—original draft preparation,M.A.S., M.S.B. and A.I.;writing—review and editing,M.M.R. and M.A.H.All authors have read and agreed to the published version of the manuscript."

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