



Epidemiology of Sub-Clinical Mastitis in Dairy Cows in Urban Areas of Chittagong, Bangladesh

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<p><i>Research Article</i></p> <p>Received : 18/10/2018 Accepted : 14/03/2019</p> <p>Keywords: Epidemiology Sub-Clinical Mastitis Cow Bangladesh Risk factors</p>	<p>Mastitis is considered to be the most devastating condition for the dairy farms of low and medium income countries due to its alarming impact on production and worldwide has been recognized as one of the most economically significant infectious diseases affecting the welfare of dairy animal. Chittagong is one of the dairy intensive regions of Bangladesh but comprehensive epidemiological studies addressing prevalence of and risk factors for subclinical mastitis (SCM) are scant. Therefore we aimed to approximate the prevalence of SCM in dairy cows along with the associated risk factors in dairy cows of Chittagong Metropolitan Area (CMA), Bangladesh. We included 114 cross breed (Holstein × Local) lactating cows, raised at 6 dairy farms in CMA between February to November, 2015 for this study. California Mastitis Test (CMT) was used to assess SCM at animal level. The overall prevalence of mastitis was 34.2% among the tested cows. Floor type, source of replacement cows, history of previous reproductive disorder, stage of lactation and cleanliness of floor was found significantly associated with SCM in univariate analysis. In random effect multiple logistic regression model, cemented floor was found to increase the odds of SCM by 5.03 times than that of brick floor. Similarly, cows with history of reproductive disorders had more risk of having SCM than that of cows without a history of reproductive disorders. Since the SCM is prevalent in the study area, the intervention strategy should focus on causal agent, improved management, frequent monitoring of SCM in milking cows with CMT and use of teat disinfectants; with minimal or no treatment with antimicrobial agents.</p>
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Introduction

Mastitis is considered to be the most devastating condition of dairy cattle. It causes remarkable production loss to the livestock industry and has been recognized as one of the most economically significant infectious disease affecting the welfare of dairy animals worldwide (Bradley, 2002; Deluyker et al., 2005; Chishty et al., 2007; Hashemi et al., 2011).

About 75-80% mastitis is of SCM type (Bradley, 2002; Biswas and Sarker, 2017). The main characteristics of SCM are- a significantly elevated Somatic Cell Count (SCC) to a level >200,000 cells/ml (Radostitis et al., 2000; McDougall et al., 2001; Bradley, 2002) without any visible

abnormalities in milk or udder tissues (Radostitis et al., 2007). SCM is 15-40 times more prevalent than the clinical one. It usually follow the clinical form of mastitis and hard to detect, which act as a continuous source of infection for herd mates. SCM reduces average 17.5 % milk production (Joshi and Gokhale, 2006) and adversely affects milk quality and quantity (Seegers et al., 2003; Swinkels et al., 2005; Halasa et al., 2007; Souto et al., 2010; Islam et al., 2012b). Singh and Singh (1994) reported more than three times production losses due to SCM, as compared to clinical mastitis (CM)(Abrahmsén et al., 2014).

The infection level of SCM in cows is accelerated with pendulous udder & mid-lactation stage (Biffa et al., 2005). SCM arising in late lactation is associated with the highest yield loss (Hortet et al., 1999; Bennedsgaard et al., 2003). Once a cow suffers from SCM, it never return to its normal milk production.

Among different indirect screening tests, California mastitis test (CMT) is considered as a simple, available, easily applied and economic diagnostic test that yield a rapid and satisfactory test result (Joshi and Gokhale, 2006). Reagents of these tests contain detergents which change the structure and conductivity of cell membrane and nucleus of somatic cells, stimulate proteolytic enzymes, and increase milk viscosity (Middleton et al., 2004).

Previous study reported 40.1% SCM in dairy cows of Ethiopia along with age, Body Condition Score (BCS), milk yield and number of parity as potential risk factors (Birhanu and Leta, 2017). Another study from North West Ethiopia stated the prevalence being 62% in cows and identified some risk factors like higher parity, >150 days in milk (DIM), housed on cemented floors (Mekonnen et al., 2017). Cow level CMT based prevalence in northern region and in sylhet, Bangladesh were reported as 15-44% (Rahman et al., 2009; Rabbani and Samad, 2010; Islam et al., 2012b; Siddiqe et al., 2015) and 51.3% (Rahman et al., 2010), respectively. SCM was reported to be 32.43% in Chittagong (Barua et al., 2014) and 65.5% in a specific upazilla (Anwara) of Chittagong (Rahman et al., 2014) by CMT. Deterioration of quality and quantity of milk, damage to udder tissue and most importantly economic loss are the ultimate results of SCM (Deluyker et al., 2005). Hence, early detection and characterization of mastitis as well as taking appropriate preventive and control measures is important. In Bangladesh, especially in Chittagong, SCM is the persisting problems in dairy industries. But in-depth study of SCM with their risk factors in the study area is limited. Hence the present study was undertaken to know the prevalence of SCM in dairy cows along with associated risk factors for the occurrence of SCM in Chittagong Metropolitan Area (CMA).

Materials and Methods:

Study Animal and Period

The study animals consists of 114 cross breed (Holstein × Local) lactating cows, raised at 6 dairy farms in CMA, Bangladesh. The study was conducted between February to November, 2015. The dairy farm list of CMA were collected from District Livestock office of Chittagong. We randomly selected six upazila of CMA and one farm from each upazila, as well. All cows were hand milked twice daily. The herd size of the farms ranged from 7 to 36 cows. Cows were reared following the cut and carry system-cows are housed, and fed forage cut-and-carried from grass land to farm without any grazing, with free access to water, and were fed a concentrate supplement after milking. To stimulate milk let-down in dairy cows calf suckling before milking was practiced. After end of each milking, calves were allowed to suck the udder without post-dipping.

Milk Sample Collection and Physical Examination of Milk Samples

Each farm was visited once and the cows were examined to rule out clinical mastitis (temperature $\leq 39.5^{\circ}\text{C}$, no signs of illness and/or inflammatory signs of the udder, and normal milk upon ocular inspection). After udder sanitation, appraisal and discarding of foremilk, around 5 ml milk from each quarter was collected and mixed all four quarter milk samples in a sterile screw capped tube. The milk were collected by an expert milk man and the hands were cleaned properly before every milking. Immediately after milk collection, samples were observed with naked eyes to detect any abnormalities in color, odor, consistency and presence of clot, blood, flakes and any other visible abnormalities.

California Mastitis Test (CMT)

The cows were tested with the CMT kit according to (Mellenberger and Roth, 2000) detect the SCM.

The CMT test result was scored from 1 to 5 according to the Scandinavian scoring system, where 1 is negative result (no gel formation), 2 is traceable (possible infection), and 3 or above indicates a positive result, where 5 has the most gel formation (Salonieni, 1995; Persson et al., 2011).

Questionnaire-Based Data Collection

Data for each animal and herd were collected using a pretested questionnaire. The information includes age, breed, number of parity, lactation stage, source of cow replacement, cleanliness and type of floor, history of reproductive diseases (For example: anestrus, dystocia, endometritis, pyometra, abortion, stillbirth etc.), and per day milk production.

Statistical Analysis

Data on risk factors and results of CMT were stored in Microsoft Excel 2013. The data were cleaned, coded and checked for integrity in MS Excel 2013 and exported to STATA-13 (StataCorp, 4905, Lakeway Drive, College station, Texas 77845, USA) to perform the statistical analysis. To find the association between explanatory variables and prevalence of sub-clinical mastitis- chi-square test was done and the level of significance was set at $P \leq 0.05$. The significant factors from univariable analysis were forwarded to multivariable logistic regression analysis considering cluster of positive animals at farm level. The model were checked for collinearity, confounding and interaction following standard statistical procedure (Dohoo and Martin, 2003). The variables were considered significant which have $P \leq 0.05$ in Wald test. The Odds ratio (OR) and 95% confidence interval were used to express the results.

Results

The study estimated SCM prevalence in dairy cows as 34.2% in intensive farming system in Chittagong, Bangladesh. The CMT scores were ranged from 1 to 3. For positive samples, most of them scored 3 and two samples scored 2 (Figure 1). The color, odor, consistency and presence of clot in the milk samples for scored 2 samples were considered positive for SCM.

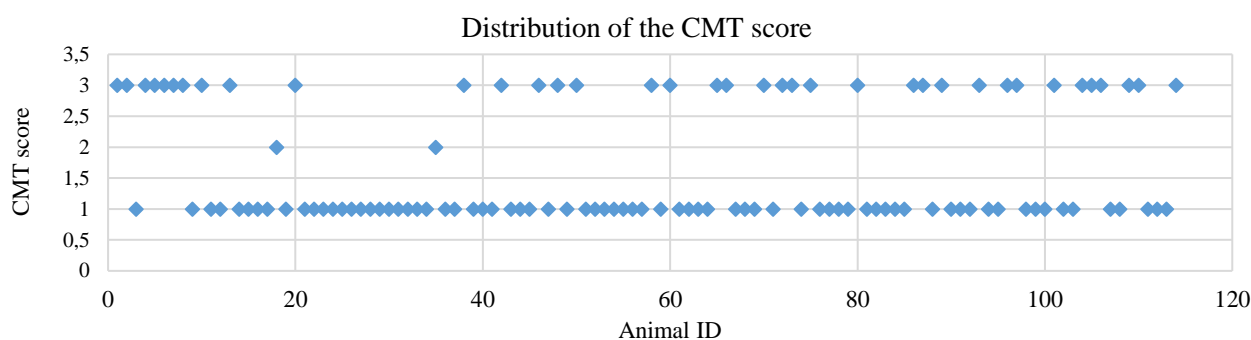


Figure 1 Distribution of CMT scores of dairy cows for SCM in CMA, Bangladesh, 2015

Table 1 Contingency tables and chi-square test conducted to evaluate the association between explanatory variables and subclinical mastitis in dairy cows in Chittagong Metropolitan Area in Bangladesh, 2015.

Variables	Categories	Positive/ Total tested	Prevalence (%)	95% Confidence Interval	Chi ²	P
Upazila	Bakolia	11/21	52	29.78-74.29	8.51	0.130
	Bayezid	12/36	33	18.56-50.97		
	Chandgaon	5/11	45	16.75-76.62		
	Halishahar	3/7	43	09.90-81.59		
	Nasirabad	6/21	29	11.28-52.17		
	Panchlaish	2/18	11	01.37-34.71		
Floor	Brick	2/18	11	01.37-34.71	5.07	0.024
	Cemented	37/96	39	28.78-49.03		
Source of animals for replacement	Own farm	28/68	41	29.37-53.77	5.79	0.055
	Other farm	2/18	11	01.37-34.71		
	Both	9/28	32	15.88-52.35		
Types of farm	Cattle	28/93	30	21.03-40.50	3.78	0.05
	Mixed species	11/21	52	29.78-74.29		
History of Reproductive disorder	Yes	12/23	52	30.59-73.18	4.13	0.042
	No	27/91	28	19.21-37.86		
Age (years)	Min-3	0/7	0	0	6.96	0.073
	>3 to <6	22/58	38	25.51-51.63		
	>6 to <8	8/31	26	11.86-44.61		
	>8	9/18	50	26.02-73.98		
Pregnancy	Fresh	23/69	33	22-46	0.06	0.807
	Pregnant	16/45	36	22-51		
BCS	≤3	32/87	37	26.69-47.80	1.08	0.299
	>3	7/27	25	10.69-44.87		
Lactation no.	<2	3/16	19	04.05-45.65	1.98	0.160
	>2	36/98	37	27.22-47.08		
Milk yield (liter)	Min-13	22/58	38	25.51-51.63	0.726	0.394
	>13	17/56	30	18.78-44.10		
Lactation period (month)	Min-2	11/30	37	19.93-56.14	10.17	0.017
	>2-5	10/36	28	14.20-45.19		
	>5-7	6/29	21	07.99-39.72		
	>7	12/19	63	38.36-83.71		
Gestation period (month)	1-4.5	32/101	32	22.78-41.69	2.51	0.113
	>4.5	7/13	54	25.13-80.78		
Cleanliness of floor	Good	10/45	22	11.20-37.09	4.75	0.029
	Poor	29/69	42	30.24-54.52		

Farms with cemented floor (39%) had significantly more events of SCM than farms having brick floor (11%) ($P < 0.05$) (Table 1). Similar significant ($P = 0.055$) relation was observed between source of replacement cow and prevalence of SCM. Prevalence was higher in pregnant cows (36%) than fresh cows (33%). The cows aged more

than 8 years showed greater prevalence (50%) of SCM than age minimum to 3 years (0%). High milk producing cows showed greater prevalence (52.94%) than medium (35.00%) and low producing cows (37.90%). Mixed farming system faced more SCM events than those having only cattle. The study also revealed a significant

association ($P < 0.05$) between the presence of history of reproductive disorder and SCM in cows. The prevalence of SCM was 52% in cows with a previous history of reproductive disorder. The study also focused on prevalence of SCM in different lactation periods of cows. The prevalence was significantly higher ($P < 0.05$) in cows with advancing the lactation time from 2nd month. Again, cleanliness of floor is an important factor behind the occurrence of SCM (Table 1).

The random effect model identified two significant factors- type of floor and history of reproductive disorder influenced the occurrence of SCM in the study population. Cemented floor increases the odds of SCM 5.03 times (95% CI: 1.10 - 23.5; $P = 0.04$) than that of brick floor. Similarly, cows with a history of reproductive disorder had more risk of having SCM than that of having no history of reproductive disorder (OR=2.6; 95% CI: 0.1 - 6.8; $P = 0.05$) (Table 2).

Table 2 Factors retained in a final mixed-effect multivariable Logistic regression model of the risk factors of subclinical mastitis in dairy cows in Chittagong Metropolitan Area in Bangladesh, 2015.

Variables	Categories	Odds ratio (95% Confidence interval)	P-value
Floor type	Brick	1	0.04
	Cement	5.03 (1.10-23.5)	
History of reproductive disorder	No	1	0.05
	Yes	2.6 (1.0-6.8)	
Insig2u	-13.04	-161 - 135	
Sigma_u	0.0014709	$1.11e^{-35}$ - $1.94e^{+29}$	
Rho	$6.58e^{-07}$	$3.77e^{-77}$ - 1	

Discussion

The overall prevalence was close to the findings reported (32.43%) from the study site earlier (Barua et al., 2014). Although the prevalence is lower than other previous reports from Ethiopia, 40.1% by Birhanu and Leta (2017) and 62% by Mekonnen et al. (2017) but within the range (19.9% - 44.8%) stated by some researchers in Bangladesh (Rahman et al., 2009; Rabbani and Samad, 2010; Islam et al., 2012a) and from other countries (25.2 to 55.2% at cow level) (Giannechini et al., 2002; Mungube et al., 2005; Joshi and Gokhale, 2006; Harouna et al., 2009; Mdegela et al., 2009; Bitew et al., 2010; Abrahmsén et al., 2014). Mastitis is a multifactorial disease that predominates the interaction between host, agent and environment (Thrusfield and Christley, 2005; Abdel-Rady and Sayed, 2009). A possible explanation of the difference in the prevalence of SCM in cows might be difference in study populations characteristics, geographical influence and others husbandry practices in the farms of different study (Mdegela et al., 2009; Sarker et al., 2013; Abrahmsén et al., 2014; Barua et al., 2014; Islam et al., 2015; Koop et al., 2016).

Replacement Stock

Significant variation was found in the presence of SCM among different source of replacement stock. Replacing cows from unknown sources is a common scenario in Bangladesh. Usually the farmers bought cows from livestock markets where animal sellers bring their animals to sell from different districts of the region even from different divisions which situated far away from the study area. The newly arrived cows in the herd may harbor different pathogen that are capable of causing mastitis and spread to healthy cows of the farms (Abrahmsén et al., 2014). Some dairy farmers maintain strict biosecurity measures in their farm. Collecting replacement stock from those farmers is helpful to maintain a good quality herd in own farms. However this is not a common practice in Bangladesh. It increases the possibility of SCM in dairy herds.

Types of Farm

Presence of sub-clinical mastitis also varies between single species farm and mixed species farm. Different species harbor different microorganisms which may cause mastitis under favorable conditions. So, presence of different species within same farm pre-disposes the exposure of variety of organisms. Besides, mixing of different species increases the chance of contamination through milker's hand and utensils. Lactation period (months) and cleanliness of floor influences the occurrence of SCM significantly in dairy farms too.

Age

Prevalence of SCM increased with the advancement of age in cows in agreement with other studies (Abdel-Rady and Sayed, 2009; Barua et al., 2014). The teat canal of older animals become more dilated or partly open condition that persist permanently due to years of repeated milking (Madut et al., 2009). This may lead to long time exposure of older animals to SCM causing microorganisms compared to younger animals. The prevalence is also increase with age as the spontaneous cure rate of SCM is low (McDougall et al., 2002). The study farms follow hand milking. Incomplete milking is usually happen in this situation that limit the self-cure rate of SCM in cows.

Stage of Lactation

In the present study, cows having gestation period more than 7 months were more susceptible to SCM is concordant to earlier findings whereas chronic mastitis, most often subclinical, is more frequent later during the lactation. The *Staphylococcus* species is one of the main causative agent of SCM due to unhygienic milking. This organism usually colonise at the teat and teat canal which may lead to increase the somatic cell counts and SCM. The use of dry cow therapy and post-milking disinfections practice are the common way to prevent this organism (Mdegela et al., 2009a). In our study farms, non-existence of dry period and post milking disinfection practice might influencing the

higher SCM in later stage. Farm owners have tendency to milk the cows for a very long periods; some times more than a year, which increase the risk of SCM (Abrahmsén et al., 2014).

History of Previous Reproductive Disorders

The history of previous reproductive disorders was found significantly associated with SCM occurrence. If a cow suffered from any reproductive disorder, it increased the chance of getting infection might be especially through reproductive tract like in case of retained placenta or endometritis which facilitate the udder to come in contact with contaminated body parts like pendulous placenta. In previous study repeat breeding was found associated with clinical mastitis and repeat breeding was increasing with increased incidence of clinical mastitis (Gustafsson and Emanuelson, 2002; Hertl et al., 2010).

Floor Type and Cleanliness

A significant risk factor for SCM is floor type and cleanliness of the floor. Cemented floor and cleanliness of the floor was acknowledged previously as an important factor for increasing the risk of sub-clinical mastitis occurrence elsewhere (Schreiner and Ruegg, 2003; Doherr et al., 2007; Sarker et al., 2013; Abrahmsén et al., 2014; Mekonnen et al., 2017). There are many pathogens that are found in dirty environment especially in the barn of animals which is capable of causing mastitis in cows. The cleanliness of the floor depends on manure management system, frequency of cleaning of barn, overcrowding, dominance of animals and easy movement facility availability for animals within the barns. Moisture, mud, and manure present in the environment of the cow are the primary sources of exposure for environmental mastitis pathogens, and hygiene scores of cows provide visible evidence of exposure to these potential sources (Schreiner and Ruegg, 2003). In this study, we were able to confirm the relationship between the increased risk of SCM and floor type. Even though we did not perform any in-depth statistics on it, a reflection is that the overall hygiene and especially the hygiene routines around milking time; and before and after calving, are might be the main reasons of the SCM occurrence in dairy cattle's of the study area. No separate grouping of cows or predetermined order of milking based on the udder health status were observed.

The study aim was to know the present situation of SCM in cows of commercial dairy farms and to contribute in improved prevention and control of SCM. We identified some possible risk factors that could be an effective guideline to build awareness among farm owners and to establish effective control strategy SCM control in the dairy farms. As well as the findings can be manipulated to reduce the SCM level in dairy herds which in turn help to raise the production and economic benefit of the farm.

Conclusion

In the present study, high prevalence of SCM in dairy cows of CMA was found by CMT. Some risk factors were found that might facilitate the occurrence of SCM in dairy cows like replacement stock, types of farm, and floor type and their cleanliness at farm level; and age, stage of lactation and history of reproductive disorders at individual animal level. Maintaining the proper hygienic condition in the dairy farm along with their milk production procedures

may help to reduce the SCM in the study area. Since this form of mastitis is an undetectable problem clinically-awareness building among the farm owners may also prevent the considerable economic losses.

Acknowledgement and Conflict of interest

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No conflict of interest.

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