Research Article

Prevalence of Subclinical Mastitis of Dairy Cows in Bijoynagar Upazila under Brahmanbaria District of Bangladesh

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Abstract | Subclinical mastitis (SCM) is an over emphasized form of mastitis causes great economical loss in dairy farms due to reduction in milk production. The aim of the study was to estimate the prevalence of subclinical mastitis in crossbred (local Zebu x Holstein Friesian or Sahiwal or Sindhi) and indigenous lactating cows. The study was carried out in different small scale dairy farms in Bijoynagar Upazila under Brahmanbaria district, Bangladesh. Bijoynagar Upazila consists of 10 unions. A total of 400 milk samples, 40 from each union, were screened for SCM by California Mastitis Test (CMT). A total of 115 cows were found positive for SCM. The overall prevalence of SCM in lactating cows was 28.75% of which 30% at Chandura union, 32.5% at Paharpur union, 20% at Potton union, 27.5% at Bodonti union, 32.5% at Isapura union, 25% at Chorislampur union, 37.5% at Horospur union, 22.5% at Chompoknagar union, 32.5% at Singerbil union and 25% at Bisnopur union. The prevalence of SCM significantly increased with age and parity of cows in Bijoynagar upazila under Brahmanbaria district, Bangladesh. This study may help the dairy farmer to take necessary precursors against SCM and recover economic losses causes by SCM.

Keywords | Subclinical mastitis, Prevalence, Breed, Age, Dairy cows

INTRODUCTION

Bovine mastitis (both clinical and subclinical) which causes by bacterial infection (mostly *Streptococcus* spp.; *Staphylococcus* spp.) in the udder and one of the most important diseases causes economic loss to the dairy farmers (Sinha et al., 2011; Pankaj et al., 2012). The clinical mastitis (CM) can be diagnosed on the basis of history and clinical findings while for the subclinical mastitis it is necessary to perform laboratory examinations. In Bangladesh most of the dairy cows are from cross breed and the overall prevalence for SCM was higher in cross breed cows (Rahman et al., 2017). The prevalence of SCM has been shown to be much greater than the clinical mastitis (Rahman et al., 2017). Kader et al. (2002) reported that the annual economic loss due to reduced milk production alone caused by SCM in Bangladesh is Tk. 122.6 million (US $ 2.11 million). However, no such accurate estimation of economic losses due to SCM in Bangladesh for the recent years (Rahman et al., 2017). The reported prevalence of SCM varied from 15.8% to 53.1% in crossbred cows (Holstein Friesian X local) in Bangladesh (Rabbani and Samad, 2010; Rahman et al., 2010). In a recent study by Islam et al. (2019) who used California Mastitis Test (CMT) to detect subclinical mastitis (SCM) and reported that the overall prevalence for SCM was 34.2 % among the tested crossbreed cows

(Holstein Friesian X local) in Chinnagong district of Bangladesh. The US national mastitis council stated that the rate of intra mammary inflammation is usually higher during the dry period than lactation period, while the rate of infection is higher at the first 75 days postpartum, than the remainder of lactation period. Mungube et al. (2004) reported that cows aged at least 8 years, with poor body condition, with at least 8 parities and in at least the eight months of lactation had a significantly higher risk for subclinical mastitis. The prevalence of SCM was highest in older cows compared to young cows (Rahman et al., 2017; Islam et al., 2019).

The SCM can be known only after laboratory examination, as there are no gross inflammatory changes in the udder tissue. Mostly all microorganisms are involved in mastitis but some factors, pendulous udder with long teats, larger size of teat orifice in high yielding cows, traumatic injuries etc. may play an important role as inciting factor of mastitis in dairy cows. Poor management, nutrition, and hygiene, are mostly associated with mastitis (Rahman et al., 2017). Once a cow suffers from mastitis it will never return to its normal milk production. Besides causing huge losses to milk production, the sub clinically affected animals remain a continuous source of infection to other herd mates. Early diagnosis of mastitisis is essential to prevent excess economic losses. The CM can easily be detected by inspection of udder and or systemic signs of inflammation, whereas diagnosis of SCM is more problematic since the milk appears normal (Rahman et al., 2017). Various physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of SCM (Pankaj et al., 2012). Although bacteriological culture of milk samples is the gold standard method for identifying mastitis but it does not provide a measure of the degree of inflammation associated with the infection (Middleton et al., 2004). Therefore, indirect tests viz. California Mastitis Test (CMT), White Side Test (WST), Surf Field Mastitis Test (SFMT) etc. can be used that are simple and do not require any complex laboratory equipment (Middleton et al., 2004).

Subclinical mastitis is not only responsible for great economic loss to the dairy industry but also acts as a source of infection for healthy milk cows, which is one of the biggest obstacles in the achievement of self-sufficiency in milk production in Bangladesh. Therefore, this study has planned to determine the prevalence of SCM so that the dairy farmers of the selected study area can take necessary precursors against SCM and recover economic losses causes by SCM.

Thus, the present research work was undertaken to estimate the effect of the stage of lactation, parity and level of milk production on the prevalence of subclinical mastitis (SCM) in Bijoynagar upazila under Brahmanbaria district.

MATERIALS AND METHODS

STUDY SITE AND SAMPLES

A cross sectional study was carried out on 400 lactating dairy cows; crossbred and indigenous lactating cows of Bijoynagar Upazila under Brahmanbria District from January 2018 to October 2018. Bijoynagar upazila consists of ten unions. A union was considered as a small territory part of study area. Forty cows were selected from each union (Table 1). Cows were crossbred between local Zebu with Holstein Friesian, Sahiwal or Sindhi, and indigenous as well. All the cows were housed loose or tied up at night and tethered during the day. Legume, other green forages, milling by-products and rice straw were fed to the cows.

Table 1: Union wise distribution of sample populations.

Union	No. of milk cows selected
Chandura	40
Isapura	40
Chompoknagar	40
Potton	40
Singerbil	40
Bisnopur	40
Paharpur	40
Horospur	40
Bodonti	40
Chorislampur	40

TEST MATERIALS

The prevalence of bovine mastitis was studied in Holstein Friesian and indigenous cattle using California Mastitis Test (CMT) kit, commercially available as Leucocytest (Synbiotics Corporation, France) (Figure 1). The Leucocytest test kit was purchased from the local market supplied by Advance Animal Science Co. Ltd. Bangladesh. It was stored at room temperature until used as per manufacturer's instructions. Leucocytest Test kit contains leucocytest reagent (1 vial of 1 litre) and leucocytest plate (1plate of 4 wells).

Figure 1: Leucocytest (CMT) test kit.

Table 2: Scoring of California mastitis test results.

Reading			Interpretation	
Aspect	Score		Infection	Corresponding to average cellular numeration($\times 10^3$/ml)
	Value	Expression		
Consistency normal or gray colored	0	-	Absent	100
Light gel disappearing after stirring or purplish gray colored	1	+/-	Infection risk by minor pathogenic	300
Light persistent gel crumbly filaments or purple gray	2	+	Subclinical mastitis	900
Immediate thickening viscous cluster at the bottom of the well	3	++	Subclinical mastitis	2700
Thick gel, consistency of egg white, color dark purple.	4	+++	Subclinical mastitis near the clinical expression	8100

DATA AND SAMPLE COLLECTION

Data were collected by using a pretested questionnaire through face to face interview of the farmers and examining cows. Samples (about 2mL/quarter) were collected from each quarter in sterilized test tubes. Immediately after collection the milk samples were subjected to California mastitis test (CMT). The test was performed as per instruction of the manufacturer. The result was interpreted as below (Table 2). If any one quarter was positive for SCM the cow was considered as SCM positive.

TEST PROCEDURE

Milk samples collected at beginning of the milking from each quarter (about 2mL/quarter) and then were placed at 4 wells of the plate as directed by the company manual. The plate was inclined above the container so that excess milk was run out. Two mL of Leucocytest reagent was added in each well. The reageant and the milk were mixed by a circular motion with the plate for a few seconds. The result was read with reference to the table of reading. The results were analyzed for significance.

STATISTICAL ANALYSIS

Data were subjected to one-way analysis of variance (ANOVA) using software SPSS (version 18) for windows (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare the means among treatments. Statements of statistical significance was based on a probability of $P < 0.05$. Results are presented as means and standard error of means.

RESULTS

PREVALENCE OF SUBCLINICAL MASTITIS

Out of 400 milk samples tested by CMT, 115 were found positive for SCM. The overall prevalence of subclinical mastitis was 28.75% (Table 3). The union-wise prevalence varied from 20- 37.5% and variation was not statistically significant (Table 4). The highest prevalence was in

Horospur union and lowest in Potton Chandura union.

Table 3: Overall prevalence of subclinical mastitis.

Test used	No. of samples tested	No. of positive cases	Overall prevalence rate
California mastitis test	400	115	28.75%

Table 4: Union wise prevalence of subclinical mastitis.

Union	No. of milk samples collected	No. of positive cases	Prevalence	Level of significance
Chandura	40	12	30%	NS (P>0.05)
Isapura	40	13	32.5%	
Chompoknagar	40	9	22.5%	
Potton	40	8	20%	
Singerbil	40	13	32.5%	
Bisnopur	40	10	25%	
Paharpur	40	13	32.5%	
Horospur	40	15	37.5%	
Bodonti	40	11	27.5%	
Chorislampur	40	10	25%	

NS: non-significant (P>0.05).

EFFECTS OF BREED, AGE, PARITY, STAGE OF LACTATION AND MILK PRODUCTION ON THE PREVALENCE OF SCM

Variations in the prevalence of SCM with the age, parity, stage of lactation and milk production are presented in Table 5. The prevalence of SCM was relatively higher in crossbred cows (29.43%) than in local indigenous cows (27.95%). Older cows (>5 years) had higher incidence of SCM (30.73%) as compared to younger cows (3-5 years). The prevalence of SCM varied with parity; which was recorded to be 18.18% at parity 1; 28.63% at parity 2; 32.63% at parity 3; 32.25% at parity 4; 13.33% at parity 5; 22.22% at parity 6; and 20.00% at parity 7. Prevalence of SCM was increased from parity 2 to 4. The prevalence of SCM was 34.14% in the early (up to 3 months), 22.85% in

the mid (4-6 months) and 25.00% in the late lactation (7 months above). The prevalence of SCM increased with the milk yield, 24.5% in cows with 1-5 L milk yield; 31.11% with 6-10 L milk yield and 37.7% with >10 L milk yield.

Table 5: Prevalence of subclinical mastitis in relation to breed, age, parity, stage of lactation and milk production.

Variables		No. of cows tested	No. of positive cases	Prevalence (%)
Breed	Cross	214	63	29.43[a]
	Indigenous	186	52	27.95[b]
Age (year)	3-5	195	52	26.67[b]
	>5	205	63	30.73[a]
Parity	1	11	2	18.18[c]
	2	234	67	28.63[b]
	3	95	31	32.63[a]
	4	31	10	32.25[a]
	5	15	2	13.33[d]
	6	9	2	22.22[bc]
	7	5	1	20[c]
Stage of lactation	Early	205	70	34.14[a]
	Mid	175	40	22.85[bc]
	Late	20	5	25[b]
Milk yield (litre)	1-5	204	50	24.5[b]
	6-10	135	42	31.11[a]
	>10	61	23	37.7[a]

a, b, c, d- means in the same row with different letters are significantly different at P < 0.05.

DISCUSSION

Mastitis is recognized worldwide as one of the economic diseases affecting dairy industry. Many dairymen do not recognize fully the tremendous losses sustained through unrealized milk production. At the subclinical level dairymen could be unaware of mastitis. Dairymen are more likely to become concerned about diseases that obviously affect the cow's health, even though such diseases may be of short duration and have less effect than mastitis on the income from the herd. This study was undertaken to determine the prevalence of subclinical mastitis (SCM), and its relationship with breed, age, parity, stage of lactation and milk production.

PREVALENCE OF SCM

In our study, 400 samples collected from different unions of Bijoynagar Upazila and examined by CMT, the overall prevalence of SCM was found to be 28.75%. A recent study reported that the prevalence for SCM was 34.2 % by California Mastitis Test (CMT) among the tested crossbreed cows (Holstein Friesian X local) in Chinnagong district of Bangladesh (Islam et al., 2019). In addition, Prodhan et al. (1996) reported 16.5% and 15.8% incidence of SCM with Whiteside test (WST) and California Mastitis Test (CMT), respectively. Sen et al. (1996) reported 14.4% and 11.5% incidence of SCM with CMT and WST, respectively. Nooruddin et al. (1997) and Rahman et al. (1997) reported 21.2% and 18.5% incidence of SCM, respectively by using modified WST. In India, Singh et al. (1982) reported 17%, Pal et al. (1989) reported 23.1%, Parai et al. (1992) reported 19.3%, Dhote et al. (1999) reported 20.7%, Pankaj et al., (2012) reported 24.45% incidence of SCM with indirect tests. Jha et al. (1993) reported 18.8% SCM in cows from Nepal.

However, higher incidence of SCM have also been reported by Singh and Baxi (1980) and was 54.0%, Motice et al. (1985) reported 54 to 81.5% and Ramachandraiah et al. (1990) reported 63.7%. Kader et al. (2002) reported 46.6% prevalence of SCM on bacteriological examination in Bangladesh. In another study by Sinha et al. (2011) reported about 42.7% prevalence of SCM on CMT test in Bangladesh. The wide variation in the incidence of SCM could be due to different study location, environmental factors, diets and the use of different diagnostic methods.

PREVALENCE OF SCM AT DIFFERENT STAGES OF LACTATION

Different prevalence rate of SCM was recorded in the early (34.14%), mid (22.85%) and late (25.00%) stage of lactation in different cows. The high rate of SCM during the early stage of lactation may be due to the high yielding cattle with enlarged status of udder are prone to infection and inflammatory process. This observation supports the report of Pal and Verma (1991) who reported an increase in prevalence of mastitis in cows producing up to 9 kg milk and lower prevalence of the disease in stages of lactation above five months, but Rahman et al. (1997) reported highest prevalence of the SCM during the 3rd month (34%) of lactation and lowest during the ≥ 5 months (2%) of lactation. In contrast higher prevalence of SCM in late stage of lactation was recorded by Islam et al. (2019) and Sinha et al. (2011) in Bangladesh. However, these variations of prevalence of the SCM among the reports might be due to seasonal variation, breed of cows and status of adapting control program.

PREVALENCE OF SCM AT THE DIFFERENT PARITY

The variable prevalence of SCM was observed at different parity, 18.18% at the first, 28.63% at the second, 32.63% at the third, 32.25% at the fourth, 13.33% at the fifth, 22.22% at the sixth and 20% at the seventh parity. In this study, highest prevalence of SCM was found in 3rd parity, which was similar by Sinha et al. (2011). Sinha et al. (2011) reported that the difference in the prevalence of mastitis between parity groups was significant and were higher at

3-4 parity. This observation also supported the reports of Rasool et al. (1985) and Devi et al. (1997) who reported an increasing incidence of SCM with advancing age (parity) and lactations.

CONCLUSION

The overall prevalence of subclinical mastitis in lactating cows was 28.75 % and the union-wise prevalence varied from 20-37.5%. The prevalence of SCM was higher in cross breed than that the local breed in this study area. The prevalence of SCM was higher in >5 years old cows than in 3-5 years old cows. The prevalence of SCM was higher in early stage than the mid and late stage of lactation in different cows. The prevalence of subclinical mastitis as determined by CMT in dairy cows of this study region was high that highlighted an important health and production problem in the small holder dairy farmers. Breed, age, number of parity and milk yield could influence the prevalence of subclinical mastitis of cows in this study area. Further study was recommended to detect the actual economic losses caused by SCM in this study area.

ACKNOWLEDGEMENTS

AUTHORS CONTRIBUTONS

All authors contributed equally.

CONFLICT OF INTEREST

There is no conflict of interest related to this publication

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