

### **ORIGINAL RESEARCH**

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# Status of subclinical mastitis in lactating cows of selected dairy farms in Bangladesh and farmers' understanding of mastitis

Authors: Amit Kumar Dey<sup>1</sup>, Chaiti Dhali<sup>1</sup>, Sompa Das<sup>2</sup>, Sukanta Chowdhury<sup>3</sup>

### Abstract

Background. Subclinical mastitis, a disease that is economically important to dairy cows, affects milk production. Management of udder health is an essential element in the process of safe milk production. Consumption of milk with a high somatic cell count (SCC) may also pose a health risk to humans. **Objectives**. We aimed to evaluate the SCC method for the detection of SCM in dairy cows and to assess farmers' knowledge, attitudes, and practices towards mastitis detection and control. Methods. A cross-sectional study was conducted to evaluate the current status of subclinical mastitis (SCM) in lactating dairy cows. The research work was also designed to assess farmers' knowledge, attitudes, and practices about bovine mastitis. A total of 320 milk samples from 80 lactating cows were collected for physical examination and tested for subclinical mastitis using SCC. Results. Out of 80 lactating cows examined, 53 (66%, 95% CI: 55-76%) cows were diagnosed with subclinical mastitis. The average cow level SCC per ml of milk was 503101. According to the farmers' selfreport, 100% of farms practiced hand milking and 100% of milkmen washed their hands before milking. The majority of farms (90%) had brick floors. Most of the farmers (70%) knew about screening for subclinical mastitis. However, they never performed screening to detect subclinical mastitis. Conclusion: The high prevalence of SCM in dairy raised significant concerns about farm management, personal hygiene and biosecurity practices. Further study is needed to identify the etiologies of SCM and its associated risk factors.

Quality Control Laboratory, Department of Livestock Services, Savar, Dhaka-1343, Bangladesh

<sup>2</sup>BCS Livestock Academy, Department of Livestock Services, Savar, Dhaka-1341, Bangladesh

<sup>3</sup>International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

### Corresponding author:

\*Sukanta Chowdhury, DVM, MS, PhD Address: International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh E-mail: sukanta@icddrb.org ORCID: 0000-0003-3053-4241 Copyright © 2023 the Author(s)

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### Introduction

Mastitis is one of the most significant diseases of dairy cows which causes a huge production loss (1, 2). Early detection of mastitis helps dairy farmers to reduce economic losses that are associated with low milk production, increased treatment costs, and discarded milk (3). Two forms of mastitis commonly occur; clinical and subclinical. The visible changes in the affected mammary gland and its secretion make clinical mastitis is difficult due to the absence of physical symptoms in the cow (4). In the subclinical form of mastitis, the milk usually appears normal visually, but there is a high somatic cell count (5).

In most dairy herds, SCM causes the greatest overall losses because it is 3-40 times more common than clinical mastitis (6). SCM is responsible for low milk production that cause more than 70% economic losses (7). In Bangladesh, the estimated economic losses due to low milk production by SCM were reported at 122.6 Taka (US  $\ddagger$  2.11) million and SCM remains a significant problem for dairy farms (8). Raw cow milk from infected udder containing Listeria, Campylobacter, Yersinia, Salmonella, Staphylococci species, and E. coli can pose a threat to human health(9).

Detection of mastitis is based on somatic cell counts (SCC) and microbiological status of the udder quarter, according to the International Dairy Federation (IDF) (10). As an important component of evaluating mastitis control, hygiene, and quality, SCC is a useful indicator of intramammary infection (IMI) (11). The SCC of the healthy quarter is usually less than 100,000 cells/ml (12, 13). The presence of microbes in the udder mainly affects milk SCC, but it is also affected by the type of microbes that affect milk SCC (14). It is essential to monitor the occurrence of subclinical mastitis in dairy herds on a regular basis. This study aimed to evaluate the SCC method for the detection of SCM in dairy cows and to assess farmers' knowledge, attitudes, and practices toward mastitis detection and control.

### Methods

A research study was undertaken at Savar Upazila using sub-areas denoted by locations of Municipality/Union Council jurisdictions from April 2021 to March 2022. The study area was located in the central part of Bangladesh. We selected 5 jurisdictions (Municipality and/or Union Council) out of one Municipality and 12 Union Councils by generating random numbers using a Microsoft Office Excel worksheet. In each of the 5 sub-areas, we chose a total of six dairy farms. A trained veterinarian and a research assistant collected milk samples from lactating cows from different farms. Samples were purchased from sellers. Dairy farm owners or managers were the research participants linked to the respective milk samples. The minimum sample size required to detect 25% prevalence with a 95% level of significance and 5% precision is 320. The sample size was calculated based on a previous study where a 25% prevalence of subclinical mastitis was detected in cows in Bangladesh (15). We included only those farms that had at least 3 lactating cows. From the chosen farms, cows that appeared to be healthy, lactating, and had no signs of clinical mastitis or had no history of antibiotic treatment within the previous 14 days of our farm visit were chosen for sample and data collection. Lactating cows with any visible signs of mastitis were excluded.

In each of the five selected unions, lactating cows were randomly selected for milk samples and data collection. We collected milk samples from each quarter of randomly selected apparently healthy dairy cows. We included both indigenous (zebu) and cross-bred cows in this study. We performed clinical examinations to assess general health conditions and udder health. We examined udders by visual inspection and by palpating the udder to identify any changes in the udder such as redness, swelling, pain, and heat. Teats and udders of selected cows were thoroughly washed and dried using a single, dry paper towel per cow with particular emphasis on the teat end. The teat end and its orifice were scrubbed vigorously with 70% ethyl alcohol using a cotton pad. To minimize the chances of environmental contamination during sampling, three or four streams of milk were discarded from the teat. To avoid debris (hair, manure, dirt) contamination, the collection vial containing 20 ml of milk from each quarter was kept at a 45° angle aseptically. The collection vial was marked as the right front (RF), left front (LF), right rear (RR) and left rear (LR) and transported immediately to the laboratory by maintaining at 4°C temperature. Initially, milk samples from each quarter were tested for the presence of flakes and clots.

The Product Quality Control Section of the Quality Control Laboratory, DLS tested milk samples for Subclinical Mastitis by Somatic Cell Counter using LACTOSCAN MCC COMBO Somatic cell counter. We collected data on each animal and herd using a structured questionnaire. Data on age, breed, number of parities, lactation stage, and per-day milk production were collected through face-to-face interviews with the farm owner/animal attendant, and also from farm records where available. We also asked them to collect information about knowledge, attitudes, and practices towards subclinical mastitis, its diagnosis, and management. We provided a unique ID on the checklist for each dairy farm. We performed descriptive analysis and summarized the entire categorical variable using frequency, percentage, and 95% CI. For numerical or continuous variables, we estimated mean and standard deviation (SD) for symmetric distribution and median and interquartile range (IQR) for asymmetric distribution.

The research review committee of the Quality Control Laboratory, Department of Livestock Services (DLS) in Bangladesh reviewed and approved the study protocol. We obtained written informed consent from the dairy farm owner/farm manager/farm workers before conducting interviews.

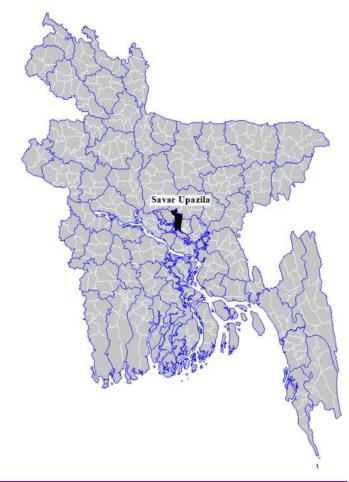
### Results

### Demographic characteristics of dairy farms

All dairy farms had cross-bred cattle. The average number of cattle per farm was 16 (range: 5-64) and the average number of lactating cows per farm was 7 (range: 2-24). The floor of the majority of farms (90%) was constructed of brick. Of the 80 enrolled lactating cows from 30 dairy farms, 35 were in the 1st lactation period, 29 were in the 2nd lactation period and 16 were in the 3rd lactation period. The mean milk production was 12 liters (range: 7-23) per cow. All cows were milked twice per day in the stanchion.

### Figure 1

Study sites for dairy cow selection and sample collection



### Prevalence of subclinical mastitis

In each of the five selected unions, a total of 16 lactating cows were randomly selected for milk samples and data collection. Area-wise lactating cow enrollment is mentioned in Table 1. A total of 80 crossbred lactating cows were selected from 30 dairy farms for milk samples and data collection. We collected a total of 320 quarter milk samples were collected from each quarter of 80 randomly chosen healthy dairy cows. Of the tested 320 samples from 80 lactating cows, 101 (32%, 95% CI: 27-37%) samples had a somatic cell count (SCC) of more than 1×106 cells/ml of milk. Out of 80 lactating cows examined, 53 (66%, 95% CI: 55-76%) cows were diagnosed with subclinical mastitis at the five study sites. The prevalence of subclinical mastitis was found highest in the Bongaon Union (Table 1). The average cow level somatic cell count (SCC) per ml of milk was 503101 (standard deviation 1227466)(range: 0-12177000).

### Table 1

Area and farm-wise prevalence of subclinical mastitis in lactating cows, April 2021 to March 2022, Savar Upazila, Bangladesh

Name of the union	Number of dairy farms	Number of lactating cows/farms sampled	Total number of dairy cows sampled	Number of lactating cows tested positive for subclinical mastitis (%)	95% CI
Birulia	6	2-3 cows/farm	16	8 (50%)	25-75%
Bongaon	6	2-3 cows/farm	16	13 (81%)	54-96%
Tatuljhora	6	2-3 cows/farm	16	10 (63%)	34-85%
Bhakurta	6	2-3 cows/farm	16	12 (75%)	48-93%
Savar	6	2-3 cows/farm	16	10 (63%)	34-85%
Total	30		80	53 (66%)	55-76%

## Farmers' knowledge, attitudes and practices towards subclinical mastitis, its diagnosis and farm hygiene

Out of 30 selected farmers, 23 (76%) farmers had awareness of subclinical mastitis and 21 (70%) farmers knew about screening for subclinical mastitis. However, all farmers reported that they did not perform screening for subclinical mastitis. A majority of farms had mastitis in their farm cows within the past 12 months. Almost all farmers knew the importance of hygienic floors for subclinical mastitis. Half of the farmers reported that they use antiseptics to clean their floors and 100% of farmers clean their udders regularly (Table 2).

#### Table 2

knowledge, attitudes and practices towards subclinical mastitis, its diagnosis and farm hygiene (n=30), April 2021 to March 2022, Savar Upazila, Bangladesh

Characteristics	Yes (%)	No (%)
Having awareness about subclinical mastitis	23 (76%)	7 (23%)
Knowing about the screening of subclinical mastitis	21 (70%)	9 (30%)
Perform regular subclinical mastitis screening	-	30 (100%)
Occurrence of mastitis within the last 12 months	20 (67%)	10 (33%)
Knowing about the importance of a hygienic floor for the reduction of mastitis occurrence	29 (97%)	1(3%)
Having farm floor constructed of brick	27 (90%)	3 (10%)
Using antiseptic to clean the floor	15 (50%)	15 (50%)
Cleaning udder regularly	30 (100%)	-
Perform hand milking	30 (100%)	-
Perform hand washing before milking by the milkman	30 (100%)	-
Washing the whole udder before milking	30 (100%)	-
Milking performed at the stanchion	30 (100%)	-

### Discussion

This study detected subclinical mastitis in 53 out of 80 milking cows examined, resulting in a prevalence of 66% subclinical mastitis in dairy farms of Savar upazila (sub-district) in Bangladesh. This result agrees with previous studies from Bangladesh by several studies that reported a prevalence of 16-57% (8, 16-19). The prevalence of subclinical mastitis in other countries including Ethiopia, Khartoum, India, and Nepal varied from 9% to 90% (20-28). There are many factors involved in the development of mastitis, primarily management, the environment, animal factors, and causative organisms.

The high prevalence of subclinical mastitis could be associated with breed susceptibility and poor hygiene and management conditions. The breed was one of the major factors related to SCC. Our study detected all subclinical mastitis in crossbred dairy cows. A previous study from Bangladesh found that the prevalence was higher in crossbred cows than in indigenous cows (29). However, our study results agree with previous studies from other countries in which the Holstein breed was shown to be more susceptible to mastitis. Previous studies found that the Holstein-Borena breed was more susceptible than the Jersey breed (30-33). Many studies showed that age and parity played roles in increasing the prevalence of SCM (29, 34, 35).

A different bacterial infection causes subclinical mastitis. Though no bacteria were isolated in this study, many studies found evidence of bacterial infection in milch cows. A

previous study from Bangladesh identified that 27 (17.1%) cows had mono-bacterial infections and 17 (10.8%) cows had mixed bacterial infections (16). Another study in India found 7 (31.8%) pure cultures from the SCM and 15 (68.2%) mixed cultures from cross-bred cows with clinical mastitis (36). Multiple bacteria such as Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae and Escherichia coli cause subclinical mastitis. Drinking unpasteurized cow milk from an infected udder containing Listeria, Campylobacter, Yersinia, Salmonella, Staphylococci species, and E. coli cause infection in humans (9).

The prevalence of subclinical mastitis can be influenced by farm management systems such as the type of floor used for lactating cows. Poor hygiene practices directly influence infection. Our study found that 97% of farmers knew the importance of hygienic floors for the reduction of mastitis occurrence. However, the prevalence of subclinical mastitis was considerably higher. A previous study reported that the highest prevalence of subclinical mastitis was recorded in poor hygiene (75.4%) and the lowest was recorded in good hygiene (19.5%). Poor hygienic practices had more chances (12.6 times) of being subclinical in terms of value than good hygienic practices. This might lead to a higher prevalence of mastitis in poor hygienic cows (37). Another study reported that the prevalence of subclinical mastitis was higher (32.69%) in lactating cows with an earthen floor, whereas the prevalence was comparatively less (23.17%) in lactating cows with a concrete floor (38).

According to the farmer's report, 100% of the dairy cows under this study were milked at stanchion which may affect the SCC in milk. There is a possibility that cows in tie stalls have lower milk SCC because they are treated individually and their health is monitored closely. Milking hygiene and mastitis detection have not been optimal with automatic milking systems (39). Mastitis is an environmental problem that is likely to be aggravated by the stall, floor, and hygienic conditions of the farm. It is well known that such material if it is not cleaned frequently, will absorb urine, feces, and other wastes from animals and their surroundings.

Knowledge of bacterial species as a bovine subclinical mastitis agent can be useful for the development of mastitis control programs. This will result in improving the health status of dairy herds. Our study found that a proportion of farmers had a high level of awareness of subclinical mastitis. Mastitis on smallholder dairy farms can be controlled with the use of udder disinfectants and improved milking hygiene. This study has several limitations. The sample of herds included in this study was probably not representative of the country as a whole. It is important to note that any subclinical mastitis case definition based on SCC may not be accurate. The prevalence of subclinical mastitis could be overestimated.

### Conclusion

Subclinical mastitis was found to be a common problem in dairy cows. Unhygienic farm environments and poor farm management may contribute to the high prevalence of subclinical mastitis. A timely diagnosis of subclinical mastitis is crucial for early effective treatment. In order to determine whether subclinical mastitis needs intervention, periodic monitoring should be conducted.

### Author contribution statement

The authors confirm their contribution to the paper as follows: **Amit Kumar Dey**: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Resources; Software; Validation; Writing-original draft. **Chaiti Dhali**: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Writing-review and editing. **Sompa Das**: Conceptualization; Formal analysis; Writing-review and editing. **Sukanta Chowdhury**: Conceptualization; Formal analysis; Methodology; Supervision; Writing review and editing.

### **Ethics statement**

The authors declare that the published work reflects an investigation and analysis carried out truthfully and completely. The research review committee of Quality Control Laboratory, Department of Livestock Services (DLS) in Bangladesh reviewed and approved study protocol. Written consent was obtained from selected dairy farmers before collecting data about their animals.

### Conflicts of interest

The authors have no conflict of interest to declare.

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### Data availability statement

Upon a reasonable request, the corresponding author can provide the study's data.

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