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*Article*

# Assessment of subclinical mastitis in milch animals by different field diagnostic tests in Barishal district of Bangladesh

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**Abstract:** This study was carried out to assess the prevalence of subclinical mastitis in milch animals by different field diagnostic tests. A total of 100 milk samples (40 cow, 40 buffalo and 20 goat) were culled to pursue this study which were subjected to physical assay and subsequently screened for subclinical mastitis by using 5 different field diagnostic tests viz. California Mastitis Test (CMT), White Side Test (WST), White Side + Dye Test (WSDT), Surf Test (ST) and Surf + Dye Test (SDT). Overall prevalence of subclinical mastitis (SCM) found in this study was 42.5%, 32.5% and 35% in cow, buffalo and goat respectively. Higher prevalence of SCM was detected in cow (47.06%) and buffalo (53.85%) aged between 3 to 5 years whereas in goat (57.15%), 2 to 3 years of age. In aspect of breed, crossbred cow (50%), Murrah buffalo (40%) and Jamunapari goat (50%) were found more affected with SCM. The prevalence of SCM was higher in cows of 3rd parity (41.18%), buffaloes of both 2nd and 3rd parity (30.77%) and goats of 2nd parity (42.86%). Animals being in mid lactation gave more positive cases (46.67% cow, 46.67% buffalo and 42.85% goat). SCM was found in higher prevalence in high yielding animals and in animals that were not being subjected to hygienic milking practice. Among the 5 diagnostic tests, SCM detection efficacy in comparison was higher by CMT. So as SCM has been found to be a major ascending risk in the area, the hygienic milk production activity in this area as a whole requires an intervention including further investigation on the etiological agents associated with prevalence of mastitis to undertake measurable control options of mastitis in the area.

**Keywords:** subclinical mastitis; prevalence; dairy animals; different filed diagnostic tests

## 1. Introduction

In Bangladesh, livestock animals which are kept for economic purpose are generally cattle, buffalo and goat. Livestock constitute an important part in the prosperity of a country, since on addition to draft power and leather, it provides manure, meat and milk to the immense majority of the people. Bangladesh holds a substantive potential for dairy development mainly due to its large livestock population coupled with the relatively congruous environment for livestock production. The livestock population census showed that Bangladesh has about 25.7 million heads of cattle, 14.8 million goats, 0.83 million buffaloes in all the regions of the country. The agricultural sector in Bangladesh, engaging 63% of the population, contributes 19.6% of the gross domestic product (GDP). The livestock sub-sector alone contributes 2.9% of the total and over14.31% of the agricultural GDP (banglapedia).

Dairy animals are biologically the most efficient cattle groups in converting feeds/roughages to milk which is a highly nutritious component for human beings. Milk is universally recognized as a complete diet due to its

essential components (Soma *et al.*, 2011). In Bangladesh, traditionally processed milk products are generally reported to be of substandard quality mainly due to inadequate dairy infrastructure such as refrigeration facility, absence of clean water and limited knowledge of the hygienic handling of milk and milk products.

Milk is designated as a complex biological fluid and by its nature, a competent growth medium for many microorganisms. Because of the specific composition, it is implausible to avoid contamination of milk with micro-organisms. Commonly found microorganisms in milk are *Bacillus cereus, Listeria Monocytogenes, Salmonella* spp., *Escherichia coli, Campylobacter jejuni, Yersinia enterocolitica, Staphylococcus* spp., *Lactobacillus*spp., *Pseudomonas* spp. etc. Therefore, the microbial content of milk is a major feature in determining its quality (Karmen *et al.*, 2008). The safety of dairy products with respect to food-borne diseases is a great concern around the world. There are some perilous milk-borne zoonotic diseases such as, tuberculosis, typhoid and paratyphoid fevers, shigellosis, cholera, *E. coli,* streptococcal infection, streptococcal food poisoning etc. This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Zelalem *et al*., 2006). Also, the composition of milk makes it an optimum medium for the growth of microorganisms that may come from the interior of the udder, exterior surfaces of the animal, milk handling equipment and other miscellaneous sources such as the air of the milking environment (Worku *et al.*, 2012). Milk has nutrients that make it suitable for the rapid multiplication of bacteria that cause spoilage. Unhygienic production, poor handling and undesirable practices such as addition of water or other substances can introduce bacteria or germs that cause spoilage (Paul *et al*., 2004).

There are several types of diseases which potentially infect and affect the wellbeing of livestock population among which mastitis is significant. Mastitis is complex disease that generally involves interplay between management practices and infectious agents, having different degrees of intensity and variations in duration and residual effects (Yien *et al*., 2014). In the complex situation of modern dairy farming, the interaction of production diseases like mastitis, their relevance with nutritional strategy, housing, environment, and the significant impact of social and attitudinal factors make prevention and control of mastitis a serious challenge.

Mastitis (inflammation of udder) is recognized worldwide as the most important and costly disease of dairy animals because it incurs great financial loss due to reduced milk yield, increased culling rates and treatment cost as well as lowering nutritive value of milk (Radostits *et al.*, 2007). Although there are two forms of mastitis- clinical and subclinical, the subclinical form of mastitis in milch cows is more important because this manner is 15 to 40 times more prevalent than the clinical form. Several causative agents and predisposing factors have been implicated in mastitis in dairy cows including bacterial, mycoplasmal and yeast pathogens, among them Coagulase Negative Staphylococci (CNS) and Coliforms are the most frequently isolated pathogens from the subclinical cow's mastitis (Islam *et al*., 2011). Animal breed, farm type, parity of cows, physiological status of animal and milk yield per animal are also found to be significant risk factors spliced with the perpetration of subclinical mastitis in dairy animal. In subclinical mastitis, the most important changes in milk include discoloration, presence of clots and the presence of large number of leukocytes (Ali *et al*., 2011). As the dairy farmers of Bangladesh aren't always sensible of the ideal contemplation to control mastitis, assessment of hygienic milk production along with early detection of subclinical mastitis by using suitable tests such as California Mastitis Test (CMT), White side + Dye Test, White Side Test (WST), Surf Test and Surf + Dye Test is very essential for its successful treatment and control.

The California mastitis test (CMT) is a simple cow-side indicator of the somatic cell count of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel. It provides a useful technique for detecting subclinical cases of mastitis (White *et al*., 2005).

The White side test is another indicator test for detecting sub-clinical mastitis in animal. The viscosity obtained in this reaction is considerably less than that of California mastitis test reaction and this test is found to be more dependent on precipitation than increased viscosity. Leucocyte nuclei were mainly responsible for the formation of the precipitate in the white side reaction and calcium chloride dispersed the precipitate formed by the leucocyte nuclei (Pandey *et al., 2010*). The white side + dye test has the same pattern to work.

Surf test is also a field test used for detecting mastitic animal that is consist of 3% household detergent (Amjed *et al.*, 2006). This test kit, when mixed with milk containing bacterial load, gives positive result by thickening the surface of milk. Surf + dye test gives positive result in this same pattern.

Several studies have been performed in Bangladesh related to prevalence of subclinical mastitis, determination of efficiency of some field tests for mastitis and assessment of hygienic milk production. Notwithstanding, there are very few comprehensive studies on prevalence of subclinical mastitis diagnosed by different tests in milch animals in Barishal district. Therefore, this study was designed to assess the hygienic milk production and

determine the prevalence of subclinical mastitis in cow, buffalo and goat in Barishal district by different field diagnostic tests in aspect of the animal's age, breed, parity, lactation, farm management etc. and to study the comparative efficiency of California Mastitis Test (CMT), White side +Dye Test, White Side Test (WST), Surf Test and Surf + Dye Test.

## 2. Materials and Methods

### 2.1. Study area and study duration

The study was carried out in different selected farms of Barisal district. The study was conducted during March 2018 to August 2018.

### 2.2. Study design and data collection

Data was collected from the farmer through questionnaire method. Personal interview of the farmers as well as history of farm will be taken.

#### 2.2.1. Study design

All the required equipments were sterilized and test kits were prepared and kept in sterilized bottles. After that farm visits were done and personal interview, history and data of the farms and farms animals were taken in the questionnaire. Thereafter, the udders were cleaned and required amount of milk was taken. The taken milk was put in the paddle where it was mixed with the individual test kits. The paddle was swirled gently in order to mix the milk and test kit. Then after a few seconds, change in milk of positive cases was observed and recorded in the questionnaire.

### 2.3. Sample Size

A total of 100 animals were examined throughout the study time. Apparently healthy 40 cattle, 40 buffalo and 20 goats were selected for sub-clinical mastitis by different test methods.

### 2.4. Collection of milk samples

The sample bottles (15 ml) were bathed with tap water, air dried, plugged and sterilized at 160◦C in the hot air oven for one hour. The bottles were then entitled indicating the dairy farm, name or number of animal and collection date. Milk samples were collected from cow, buffalo and goats after washing with clean water along with antiseptic wash of teats with 70% ethanol and desiccated by tissue paper. Two streams of milk were discarded and then 8 ml of milk were taken aseptically into labeled sterilized test tubes with rubber cup at the time of morning milking.

### 2.5. Detection of subclinical mastitis

Immediately after collection of milk, samples were subjected to CMT, WST, ST, White side + dye test and Surf + Dye Test as detailed below:

#### 2.5.1. California mastitis test (CMT)

CMT kit (CHEIL BIO Co. Ltd.) usedin this study was composed of Alkyl Aryl sulfonate (3%), sodium hydroxide (1.5%) and bromocresol purple (1:10,000) as an exponent. For conducting the CMT, a shallow half black paddle having four cups was used and was washed after each use. About 8 ml milk (2 ml from each teat) was taken hygienically and then drawn from bottle into the cup and a reckoned equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was done by gentle circular vogue of the paddle in a horizontal plane. The reaction evolved almost right away with milk containing a high concentration of somatic cells. The summit of reaction was attained within 10 seconds and scored.

#### 2.5.2. White side test (WST)

The WST reagent solution is comprised of 4% sodium hydroxide (NaOH pellets, Qualikems Fine Chemical Pvt. Ltd.) and distilled water. In order to perform the test, a shallow half black paddle having four cups was used and was cleaned after uses every time. About 1 ml milk was poured into the cup from the bottle and an estimated five drops of WST reagent was squirted from a polyethylene wash bottle. Mixing was done by slow circular motion of the paddle in a horizontal plane. The reaction showed up immediately with milk containing a high concentration of somatic cells. The peak of reaction was attained within 30 seconds and scored right away.

**2.5.3. White side + Dye test (WSTD)**
The reagent solution was composed of 4% sodium hydroxide modified with bromocresol purple 0.01% (Bromocresol Purple Powder, Qualikems Fine Chemical Pvt. Ltd.) in the laboratory. The test was obtained and interpreted following the same procedure as narrated above for WST. The apex of reaction was gained within 30 seconds and immediately scored.

**2.5.4. Surf test**
Reagent solution for surf test was formed of 3% household detergent (Uniwash, Lever Bangladesh Ltd.). The test was performed and scored similarly as the method described by (Amjed *et al.,* 2006). A shallow half black paddle having four cups was used and was rinsed after each use. About 1 ml milk was drawn from collection bottle into the paddle cup and 5 ml reagent was taken from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane. The reaction developed forthwith with milk depicting a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and scored right away.

**2.5.5. Surf + Dye test**
The reagent solution of 3% household detergent was modified with bromocresol purple 0.01% (Bromocresol Purple Powder, Qualikems Fine Chemical Pvt. Ltd.) in laboratory. The test was accomplished and interpreted in accordance with the same technique followed in Surf test. The peak of reaction was behold within 30 seconds and immediately scored.

**2.6. Result analysis**
Prevalence was calculated as the number of positive cases of subclinical mastitis. The association of age, breed, parity, stage of lactation, daily milk yield and type of floor. The result of comparative efficacy of different tests was also determined by the calculation of positive cases of SCM that are being found among the all tested samples.

**3. Results**
**3.1. Overall prevalence**
Of 100 samples collected from different areas of Barisal in Bangladesh and were examined by CMT, WST, WSDT, ST and SDT, of which yielded an overall prevalence of SCM was 37%. Among the 40 cow, 40 buffalo and 20 goat samples, 17(42.50%), 13(32.50%) and 7(35%) samples respectively were found positive to the tests.

**3.2. Age wise prevalence**
In cow, among the 17 positive cases, SCM was recorded as 3(17.64%), 8(47.06%), 4(23.53%) and 2(11.76%) respectively at the age group of 2 to 3 years, 3 to 5 years, 5 to 7 years and 7 to 9 years. In case of buffalo, among the 13 positive cases, SCM was recorded as 4(30.77%), 7(53.85%), 1(7.69%) and 1(7.69%) respectively at the age group of 2 to 3 years, 3 to 5 years, 5 to 7 years and 7 to 9 years. In the 7 positive cases of goat, SCM was recorded as 1(14.29%), 4(57.15%) and 2(28.57%) respectively at the age group of 1 to 2 years, 2 to 3 years and 3 to 5 years (Figure 1).

**3.3. Breed wise prevalence**
A total of 7(35%) local breed cow out of 20 were found to be positive where 10(50%) cross breed out of 20 tested animals gave positive results. In buffalo, among 30 local breed animals 9(30%) animals found to be positive with SCM tests and among 10 murrah breed animals 4(40%) animals gave positive result. In case of goat, SCM was found more prevalent (50%) in Jamunapari breed than Black Bengal breed (33.33%) (Figure 2).

**3.4. Parity related prevalence**
In the 17 positive cases, the prevalence of SCM in cows was recorded 11.76%, 29.41%, 41.18%, and 17.65% during the parity numbers 1, 2, 3 and 4 respectively. The parity wise prevalence of SCM in 13 buffalo was recorded as 23.07%, 30.77%, 30.77% and 15.38% during the parity numbers 1, 2, 3 and 4 respectively. Among the 7 positive cases of goat, 14.26%, 42.86%, 28.57% and 14.26% prevalence was recorded during 1$^{st}$, 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ parity, respectively (Figure 3).

### 3.5. Lactation related prevalence

This study showed 41.18%, 46.67% and 37.50% SCM prevalence in early, mid and late lactation respectively. In case of buffalo, 26.67%, 46.67% and 20% animals were found to be positive of SCM in early, mid and late lactation, respectively. Among the goats, 37.5%, 42.85% and 20% prevalence of SCM were found in early, mid and late lactation respectively (Figure 4).

### 3.6. Milk production basis prevalence

Current study showed 41.67%, 45% and 37.5% SCM prevalence in animals which produces .5 to 1 liter, 1.5 to 3 liter and 3 to 5 liter of milk respectively. The incidence of SCM in buffalo was 30.77%, 35% and 28.57% in milk yield group of 0.5 to 1 liter, 1 to 2 liters and 2 to 3 liters respectively. The incidence of SCM in goat was 42.86% and 16.67% in milk yield group of 0.5 to 1 liter and 1 to 1.5 liters respectively (Table 1).

### 3.7. Hygienic milking wise prevalence

According to washing of udder and teats before milking, the study showed higher prevalence (48.28%) in cow which were not cleaned before milking and comparatively much less prevalence (27.27%) in the animals which were cleaned before milking. According to washing of udder and teats before milking in buffalo, the study showed higher prevalence (40%) in cow which were not cleaned before milking and comparatively less prevalent (20%) in the animals which were cleaned before milking. In case of goat, according to washing of udder and teats before milking, the study showed higher prevalence (35.29%) in cow which were not cleaned before milking and comparatively less prevalence (33.33%) in the animals which were cleaned before milking (Figure 5).

### 3.8. Different field tests result

To compare the diagnostic efficacy of CMT, WST, WSDT, ST and SDT, 40 cows with 160 quarters, 40 buffaloes with 160 quarters and 20 goats with 40 quarters were randomly selected and subjected to the above tests. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 40 cows is depicted in the following table. After adding of CMT, WST, WSDT, ST and SDT kits with the milk samples, the animal wise incidence was 42.50%, 40%, 40%, 35% and 35% respectively, whereas quarter wise incidence was 37.5%, 35%, 35%, 30% and 30%, respectively. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 40 buffaloes is depicted in the following table. After adding of CMT, WST, WSDT, ST and SDT kits with the milk samples, the animal wise incidence was 32.50%, 27.50%, 27.50%, 25% and 25% respectively, whereas quarter wise incidence was 28.13%, 25.62%, 25.62%, 23.13% and 23.13%, respectively. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 20 goats is depicted in the following table. After adding of CMT, WST, WSDT, ST and SDT kits with the milk samples, the animal wise incidence was 35%, 35%, 35%, 30% and 30%, respectively, whereas quarter wise incidence was 27.5%, 27.5%, 27.5%, 25% and 25%, respectively (Table 2).

**Table 1. Milk production wise prevalence of subclinical mastitis in animal. High yielding animals showed more susceptibility to SCM than low milk producing animals.**

| Species | Daily milk yield | No. of animals | No. (%) of positive cases |
|---|---|---|---|
| Cow | 0.5-1.5 liters | 12 | 5 (41.67) |
| | 1.5-3 liters | 20 | 9 (45) |
| | 3-5 liters | 8 | 3 (37.50) |
| Buffalo | 0.5-1 liters | 13 | 4 (30.77) |
| | 1-2 liters | 20 | 7 (35) |
| | 2-3 liters | 7 | 2 (28.57) |
| Goat | 0.5-1 liter | 6 | 1 (16.67) |
| | 1-1.5 liters | 14 | 6 (42.86) |

**Table 2. Comparative study on the prevalence of subclinical mastitis in animals by different tests. Among the 5 diagnostic tests, SCM detection efficacy in comparison was higher by CMT in case of all the species and then by WST, WSDT, ST and SDT in the order.**

| Species | Name of the tests | No. of animals | | | No. of quarters | | |
|---|---|---|---|---|---|---|---|
| | | Tested | Positive | Incidence | Tested | Positive | Incidence |
| Cow | CMT | | 17 | 42.50% | | 60 | 37.5% |
| | WST | | 16 | 40% | | 56 | 35% |
| | WSDT | 40 | 16 | 40% | 160 | 56 | 35% |
| | ST | | 14 | 35% | | 48 | 30% |
| | SDT | | 14 | 35% | | 48 | 30% |
| Buffalo | CMT | | 13 | 32.50% | | 45 | 28.13% |
| | WST | | 11 | 27.50% | | 41 | 25.62% |
| | WSDT | 40 | 11 | 27.50% | 160 | 41 | 25.62% |
| | ST | | 10 | 25% | | 37 | 23.13% |
| | SDT | | 10 | 25% | | 37 | 23.13% |
| Goat | CMT | | 7 | 35% | | 12 | 30% |
| | WST | | 7 | 35% | | 11 | 27.5% |
| | WSDT | 20 | 7 | 35% | 40 | 11 | 27..5% |
| | ST | | 6 | 30% | | 10 | 25% |
| | SDT | | 6 | 30% | | 10 | 25% |

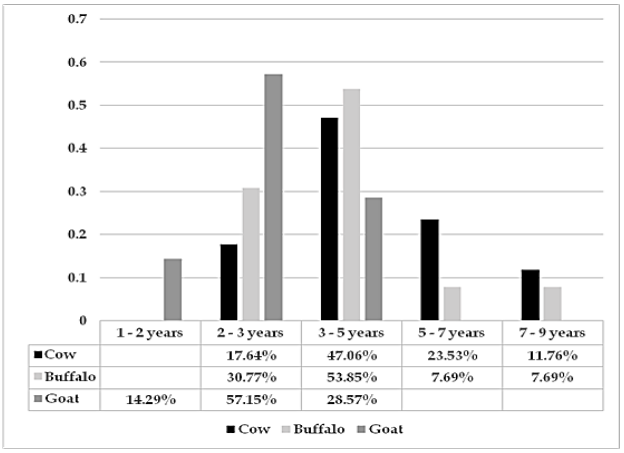


**Figure 1. Age wise prevalence of SCM in animals. Higher prevalence of SCM was detected in cow (47.06%) and buffalo (53.85%) aged between 3 to 5 years whereas in goat (57.15%), 2 to 3 years of age.**

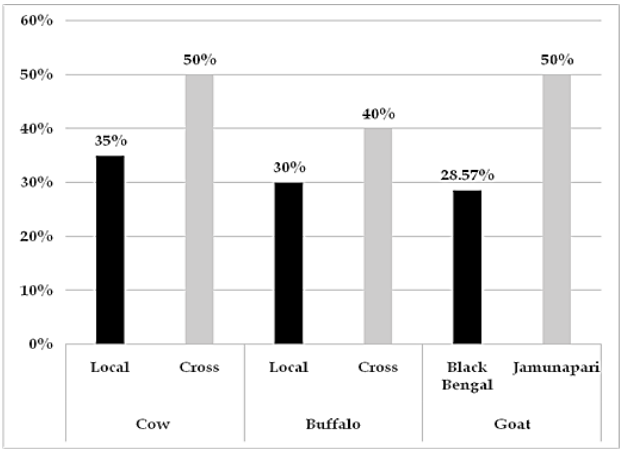


**Figure 2. Breed wise prevalence of SCM in animals. crossbred cow (50%), Murrah buffalo (40%) and Jamunapari goat (50%) were found more affected with SCM than local cow (35%), local buffalo (30%) and Black Bengal goat (28.57%).**

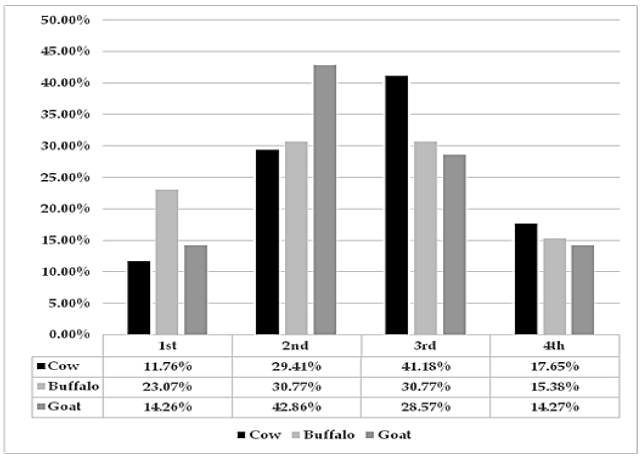


**Figure 3. Parity related SCM prevalence in animals. prevalence of SCM was higher in cows of 3rd parity (41.18%), buffaloes of both 2nd and 3rd parity (30.77%) and goats of 2nd parity (42.86%).**

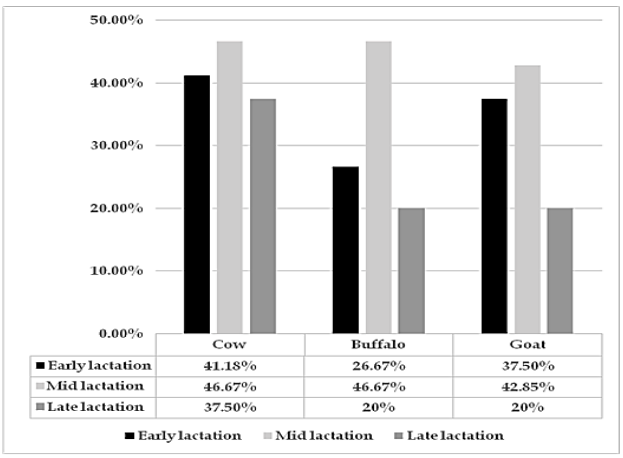


**Figure 4. Lactation related prevalence of SCM in animals. Animals being in mid lactation gave more positive cases (46.67% cow, 46.67% buffalo and 42.85% goat) than animals of early and late lactation.**

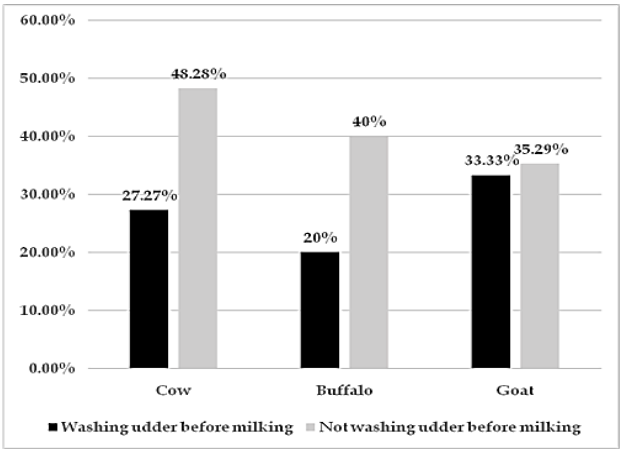


**Figure 5. Prevalence of SCM in animals in relation to hygienic management. Animals were more prone to SCM where washing udder before milking did not get appropriate concern.**

## 4. Discussion

Apart from clinical form, SCM is inflicting great economic losses (Dhakal *et al*., 2007). This is mainly attributed to severe drop in milk production, decrease in milk quality, increased veterinary expenses due to excessive use of medications, increased labor costs and increased culling rate and decrease reproductive efficiency in high producing animals (Bansal *et al.*, 2004). In addition, potential health risks were also encountered as milk from affected animals may harbor pathogenic organisms to human, increased risk of residues in the milk and consequently the possibility of public health hazards (Bilal *et al*., 1999).

This study showed that in case of cow, among 40 samples 17 (42.5%) no. of samples were found positive. Kader *et al.* (2002) also reported 46.6% SCM positive test in his study. Among 40 buffalo samples 13 (32.5%) no. of SCM positive cases had been found. Ali *et al.* (2011) reported 44% SCM positive cases in his study. In goat, 20 animals were examined where 7(35%) animals come out with positive test result and Islam *et al.* (2012) showed 38.9% positive cases.

Figure 1 showed that, in cow, among the 17 positive cases, SCM was recorded as 3(17.64%), 8(47.06%), 4(23.53%) and 2(11.76%) respectively at the age group of 2 to 3 years, 3 to 5 years, 5 to 7 years and 7 to 9 years. This study showed highest prevalence of SCM is at the age of 3 to 5 years which is 47.06% and lowest prevalence is at 7 to 9 years of age which is 11.76%. Islam *et al.* (2011) reported highest prevalence (27.94%) at the age of 5 to 7 years and lowest prevalence (18.19%) at the age of 8 to 10 years. In case of buffalo, among the 13 positive cases, SCM was recorded as 4(30.77%), 7(53.85%), 1(7.69%) and 1(7.69%) respectively at the age group of 2 to 3 years, 3 to 5 years, 5 to 7 years and 7 to 9 years. From this study it has been shown that animals of lower age are more susceptible than animals older than 5 years of age. Salvador *et al.* (2012) also reported that younger buffalo are more susceptible than older animals.

Figure 1 also depicted, in the 7 positive cases of goat, SCM was recorded as 1(14.29%), 4(57.15%) and 2(28.57%) respectively at the age group of 1 to 2 years, 2 to 3 years and 3 to 5 years. Here, 2 to 3 years of aged group is the highest prevalent group. Sood *et al.* (2008) reported that 4 to 5 years of age group is the highest prevalent group in SCM.

In Figure 2 we can see that 7(35%) local breed cow out of 20 were found to be positive whereas 10(50%) cross breed out of 20 tested animals gave positive results. So, the prevalence of SCM was higher in cross breed. Islam *et al.* (2011) also found higher prevalence in cross breed than local breed cows in their study. In buffalo, among 30 local breed animals 9(30%) animals found to be positive with SCM tests and among 10 murrah breed animals 4(40%) animals gave positive result. SCM seemed to be more prevalent in murrah breed than local breed animals. Srinivasan *et al.* (2013) also found the same results in their study. In case of goat, SCM was found more prevalent (50%) in Jamunapari breed than Black Bengal breed (33.33%) which is similar to the report by Nath *et al.* (2014).

According to Figure 3, in the 17 positive cases, the prevalence of SCM in cows was recorded in 11.76%, 29.41%, 41.18%, and 17.65% during the parity numbers 1, 2, 3 and 4 respectively. Rahman *et al.* (1997) showed the prevalence of SCM in cows was recorded in 18.75%, 22.22%, 29.55% and 26.66% during the parity numbers 1, 2, 3 and 4 respectively. The parity wise prevalence of SCM in 13 buffalo was recorded as 23.07%, 30.77%, 30.77% and 15.38% during the parity numbers 1, 2, 3 and 4 respectively. Srinivasan *et al.* (2013)

showed the prevalence of SCM in cows was recorded in 17.94%, 20.58%, 23.07% and 32.50% during the parity numbers 1, 2, 3 and 4 respectively which is quite similar to the current study. Among the 7 positive cases of goat, 14.26%, 42.86%, 28.57% and 14.26% prevalence were recorded during 1st, 2nd, 3rd and 4th parity respectively whereas, Gabriwahid *et al.* (2012) reported 18.33%, 34.78%, 32.13% and 14.55% prevalence of SCM in 1st, 2nd, 3rd and 4th parity respectively.

By the given result of Figure 4, this study showed 41.18%, 46.67% and 37.50% SCM prevalence in early, mid and late lactation respectively. Deng *et al.* (2014) reported 56.52%, 64.71% and 57.45% in early, mid and late lactation respectively. In case of buffalo, 26.67%, 46.67% and 20% animals were found to be positive of SCM in early, mid and late lactation respectively. Srinivashan *et al.* (2013) reported 17.44%, 19.29% and 44.44% prevalence in early, mid and late lactation respectively. Among the goats, 37.5%, 42.85% and 20% prevalence of SCM were found in early, mid and late lactation respectively., Gabriwahid *et al.* (2012) found higher prevalence in late lactation than early.

In Table 1, current study showed 41.67%, 45% and 37.5% SCM prevalence in cows which produce .5 to 1.5 liters, 1.5 to 3 liters and 3 to 5 liters of milk, respectively. Islam *et al.* (2011) found quite similar prevalence of SCM in cow in their study. The incidence of SCM in buffaloes were 30.77%, 35% and 28.57% in milk yield group of 0.5 to 1 liter, 1 to 2 liters and 2 to 3 liters, respectively. Medium yielder groups found to be highest prevalent of SCM. Ghosh *et al*. (2004) reported that the prevalence of SCM was found to be lowest in medium yielders group and the incidence of SCM was 60, 47.62 and 88.89 per cent in high, medium and low milk yielder, respectively. The incidence of SCM was 42.86% and 16.67% in milk yield group of 0.5 to 1 liter and 1 to 1.5 liters respectively. Kurundkar (2003) reported high prevalence of SCM in high yielding goat.

According to washing of udder and teats before milking, in Figure 5, the study showed higher prevalence (48.28%) in cow which were not cleaned before milking and comparatively much less prevalence (27.27%) in the animals which were cleaned before milking. Deng *et al.* (2014) showed same comparison in their study. According to washing of udder and teats before milking in buffalo, the study showed higher prevalence (40%) in cow which were not cleaned before milking and comparatively less prevalence (20%) in the animals which were cleaned before milking. Deng *et al.* (2014) showed same comparison in their study.

Ghulam *et al.* (2010) also reported higher prevalence in animals that are not being washed before milking. In case of goat, according to washing of udder and teats before milking, the study showed higher prevalence (35.29%) in cow which were not cleaned before milking and comparatively less prevalence (33.33%) in the animals which were cleaned before milking. Deng *et al.* (2014) showed same comparison in their study. Rabbani *et al.* (2010) also reported higher prevalence in animals that are not being washed before milking.

According to Table 2, to compare the diagnostic efficacy of CMT, WST, WSDT, ST and SDT, 40 cows with 160 quarters, 40 buffaloes with 160 quarters and 20 goats with 40 quarters were randomly selected and subjected to the above tests. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 40 cows is depicted in the following table. According to CMT, WST, WSDT, ST and SDT the animal wise incidence was 42.50%, 40%, 40%, 35% and 35% respectively, whereas quarter wise incidence was 37.5%, 35%, 35%, 30% and 30%, respectively. Dasohari *et al.* (2018) showed according to CMT, WST, SFMT, SCC and cultural examination, the animal wise incidence was 86.66%, 73.33%, 70%, 80% and 83.33%, respectively, whereas quarter wise incidence was 48.69%, 38.26%, 36.52%, 46.08% and 51.30%, respectively. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 40 buffaloes is depicted in the following table. According to CMT, WST, WSDT, ST and SDT the animal wise incidence was 32.50%, 27.50%, 27.50%, 25% and 25% respectively, whereas quarter wise incidence was 28.13%, 25.62%, 25.62%, 23.13% and 23.13%, respectively. Iqbal *et al.* (2006) showed according to CMT, WST, SFMT, SCC and cultural examination, the animal wise incidence was 40.56%, 33.76%, 32.12%, 29.76% and 29.98%, respectively, whereas quarter wise incidence was 31.12%, 28.78%, 28.12%, 26.34% and 26.11%, respectively. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests in 20 goats is depicted in the following table.

According to CMT, WST, WSDT, ST and SDT the animal wise incidence was 35%, 35%, 35%, 30% and 30% respectively, whereas quarter wise incidence was 27.5%, 27.5%, 27.5%, 25% and 25%, respectively. Islam *et al.* (2012) showed according to CMT, WST, SFMT, SCC and cultural examination, the animal wise incidence was 39.88%, 38.34%, 37.66%, 33.54% and 33.11%, respectively, whereas quarter wise incidence was 41.69%, 37.26%, 36.52%, 34.08% and 33.30%, respectively.

The above results showed that CMT gives more positive results than rest of the tests. Sharma *et al.* (2008) supports the previous results of the study.

## 5. Conclusions

The overall prevalence of subclinical mastitis in dairy animals in Barishal district of Bangladesh was 37%. What is more, the prevalence of subclinical mastitis in cow, buffalo and goat is increasing day by day than that of previous days. From the results, it can be apparent that certain risk factors including age, breed, parity, lactation, hygienic practices and daily milk yield may have been accountable for increased prevalence of subclinical mastitis in lactating animals. Lack of implementation of the routine mastitis test program, prevention and control practices by all of the farms and the preponderance of the predisposing factors noted are the principal reasons for the observed prevalence of subclinical mastitis in the milk shed. Various bacterial agents associated with subclinical mastitis in cows are getting chances to grow in milk due to lack of awareness of the inhabitant in the study area. Besides, the animal health service delivery necessity to focus on regular screening of dairy animals for subclinical mastitis and treating of the cases both lactation and dry period, and provision of advice to cull chronically infected animals. Hygienic management might help in the reduction of the prevalence of subclinical mastitis. For early detection of subclinical mastitis. CMT or all the other field diagnostic test methods can be conducted on a regular basis as a control measure. To identify the affected animal, the best therapeutic management could be used to get rid of these problems and furthermore, highest care must be given to produce good quality milk and milk products.

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## Conflict of interest

None to declare.

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