Abstract

Mastitis is one of the most common production disease found all over the world. The study was aimed to investigate the prevalence of subclinical mastitis at Muktagacha, Mymensingh , during the period of 12 October 2019 to 12 December 2020. Total of 60 lactating cows were examined to collect subclinical mastitis related information by using a formal questionnaire. Subclinical mastitis was diagnosed on the basis of clinical examinations and confirmed by California Mastitis Test. The prevalence of mastitis was 36.7% (22) in the studied area. Among 22 positive cases 23.3% (14) were affected with mild, 11.7% (7) with moderate and 1.7% (1) were severely affected. The prevalence of mastitis in Sahiwal cross, Friesian cross, Local Zebu were 32.0%, 50.0% and 26.7%, respectively. The prevalence of mastitis in 3-4, 5-6, 7-8 years old cows were 33.3%, 36.8%, and 40.0% subsequent. The occurrence of subclinical mastitis in the cows of parity 1st - 2nd, 3rd - 4th and 5th - above was and 28.6%, 35.0% and 58.3% respectively. The cow yield 1-5 liter of milk found more prone to subclinical mastitis. Among the different quarters of the cows, left rear quarter was found more susceptible to subclinical mastitis.

Keywords: Subclinical Mastitis, California Mastitis Test, Prevalence

Chapter 1: Introduction

Mastitis is the inflammation of mammary gland which is characterized by physical, chemical, pathological, microbiological changes in udder and milk. Chemical changes include discolor, clots, leukocytes and pathological changes including swelling, heat, pain, and edema (Constable, et al., 2017). At any time, up to 50% of the cows may be infected in one or more quarters (Ziv, 1992). Due to this condition, there are decreases in the production of lactose, fat, SNF, and casein. Whey proteins, Sodium, chloride, pH, and free fatty acid are increased (De Graves et al, 1993; Heath, 1994) in mastitis milk.

On the basis of etiology, it is classified into i) Contagious ii) Environmental mastitis. Both types of mastitis again classified into 2 types i) Subclinical mastitis (SCM) ii) Clinical mastitis (CM) (Rollin et al, 2015)

Sub clinical mastitis is the most, is 15-40 times more prevalent than the clinical form. In SCM, milk appears normal with no visible abnormalities. SCM usually precedes the clinical form and constituted a reservoir of microorganisms. Staphylococci have been reported as the most common pathogen group associated with SCM.

Clinical mastitis is classified into 2 categories. i) acute ii) chronic mastitis. Acute mastitis is categorized into three on the basis of clinical sign and severity (UHB, 2019). Grade I, Mild, includes changes in milk (clots, curds, bloody, watery). Grade II, Moderate, includes changes in milk and udder (Red, swollen, Painful & hot). Grade III, Severe, includes Changes in milk, changes in udder and systemic changes (Fever, Anorexia, Dullness, Depression, recumbency). *Staphylococcus aureus* a coagulase positive staphylococci has been found as the most common bacterial pathogen associated with CM whereas coagulase negative staphylococci has been reported as the most prevalent in SCM in goats.

Economically, it is one of the most devastating diseases affecting dairy animals worldwide. The economic losses are attributed to reduced milk production, discarded/poor quality milk, early culling, cost of veterinary services, decrease export of milk as well as milk products and the extra cost of management. In Bangladesh, 122.6 million Taka per year losses due to reduce milk yield (Kader et al. 2003)

Considering all the facts present study was undertaken to investigate the prevalence of SCM with different parameters like milk production, parity etc.

Chapter 2: Materials and Methods

2.1.   Research area and duration

The present study was conducted by Veterinary Surgeon along with me in Upazila livestock office, Muktagacha, Mymensingh, in response to my internship research topic. The field investigation was done at small scale dairy farm in Muktagacha, Mymensingh. I, including a teacher, made a questionnaire for collection of data.

2.2.   Selection of Animals

A total of sixty cows of dairy farms were selected randomly to conduct the present study. Sixty cows of different breeds, ages, parities and lactation stages were investigated to study the prevalence of mastitis.

2.3.   Data collection

Data was collected through interviewing with farmers who was come to Upazila livestock office. The cows in the farms were examined for the selected variables and the data were recorded using the specific form prepared for management of mastitis. The information which was not observable during farm visit was collected by asking the farmers (ages, parity, lactation stage, milk yield). Examination of the udder and milk was performed to identify the sub clinical and clinical form of mastitis in the cows. Sub-clinical mastitis was diagnosed by using California Mastitis Test (CMT). Udder and CMT score were recorded according to the procedure of National Mastitis Council (1997).

2.4. Milk quality

Table 1: Determination of quality of Milk

Reaction	Decision	Somatic cell counts/mL	Polymorph nuclear leukocyte (%)
Mixture remain liquid state	-	0 ~ 200,000	0~25
A slight slime formed , Trace reactions tend to disappear with continued movement of the fluid	+,-	150,000~500,000	30~40
A distinct slime but with no tendency toward gel formation.	+	400,000 ~1,500,000	40~60
The mixture thickens with gel formation, collected to center of cups when rotate the test paddle.	++	800,000~ over 5,000,000	60~70
The mixture thickens with gel formation, icky adhere to base of cups. There is a central peak which remains projecting above the main mass after then motion of the paddle has been stopped. Viscosity is greatly Increased so that there is a tendency for the mass to adhere to the bottom of the cup.	+++	Over 5,000,000	70~80
Indicated by a contrasting deeper purple color. It's the result of inflammation or in drying-off of the mammary gland.	Alkali milk	Over 5,000,000	Over 80
Bromocresol purple is distinctly changes yellow color at pH 5.2	Acidic milk	Over 5,000,000	Over 80

Sub clinical mastitis associated with some organisms increase the somatic cell count (SSC) as 357,000 to 1,151,000 cells/ml. The somatic cell count and N-acetyl-beta-D-glucosaminidase activity were significantly higher than uninfected. The plasminogen activator and plasmin activities were significantly higher whereas plasminogen activity and the ratio PLG: PLwas significantly lower in the infected gland. Concentration of Ca^{2+} did not differ, whereas Ca^{2+} activity was significantly lower and proteose peptone concentration was 2.4 times as high in the infected glands. Curd yield was significantly lower in the infected glands.

2.5.   Reagent preparation, conducting CMT for mastitis

Rapid indirect test, CMT is used to detection of SCM early. The entire CMT concentrate bottle (Original schalm CMT, California Mastitis Test, Techni Vet, USA.) content was added to ten bottle of water for making one gallon of working solution according to manufacturer's instruction.). For performing CMT, 2 ml of milk was collected in individual cups by hand milking when the paddle held nearly horizontal position. An equal amount of the CMT reagent was added to the milk. The paddle was then rotated in a circular motion thoroughly to mix the contents. The mixture was scored within 10 seconds while rotating the paddle. The paddle was rinsed properly with water before being used for the next test. The CMT test results were classified as either negative or positive depending on the intensity of reaction. The result of the CMT was scored and recorded on the basis of gel formation. Samples with a CMT score of 1 considered as negative, while those with CMT scores of 2, 3 and 4 were considered as positive .

Description of visible change	Interpretation	Scores
Mixture remains liquid, no slime or gel formation	Negative	1
Mixture becomes slimy or gel like. It seem to best advantage by tipping the paddle back and forth, while observing mixture as it flows over the bottom of the cup	Suspicious(mild)	2
Mixture distinctly forms a gel	Positive(moderate)	3
Mixture thickens immediately tends to form jelly. Swirling the cup moves the mixture in towards the center exposing the outer edges of the cup	Positive(severe)	4

2.6.   Clinical diagnosis of mastitis

Mastitis was diagnosed by physical examination of the cows , visual examination of milk and palpation of udder, and examination of milk by CMT for confirmation. Findings of udder palpation were scored into 1, 2, 3 and 4 for no swelling or pain in udder, swollen ventral quarter, generalized swollen quarter and, swollen and painful udder, respectively. Milk was scored as 1 = normal, 2 = flacks / clots otherwise normal milk, 3 = little / no milk, moderately abnormal color and 4 = no normal milk, watery, serum or blood. CMT scores were 1, 2, 3 and 4 . Positive cases of mastitis were expressed as mild, moderate and severe on the basis of examination findings

Table 3: Classification of mastitis based on milk, udder and cow examination

Mastitis category	CMT Score	Udder Score	Cows
Mild	1	1	Normal
Moderate	2	2	Normal
Severe	3-4	3-4	2 or more abnormal physical examination parameters

2.7. Statistical analysis

The data was obtained from a written questionnaire which is coded, tabulated, analyzed according to objective, prevalence of mastitis in small scale dairy farm at Muktagacha, Mymensingh.

Note: Prevalence was calculated according to the formula given by Thrusfield (2005). Prevalence = No. of animals with the disease/No. of animals at risk.

Chapter 3: Results and Discussion

The number, percentages and stages of mastitis in the selected areas are placed in Table 4. A total number of 60 dairy cows were included. 36.7% is the total mastitis cows where 23.3% is in mild condition, 11.7% is moderate and 1.7% is severe mastitis case.

Table 4 : Number, percentages and stages of mastitis in the selected areas

Total no. examined	No. affected	Mastitis %	Stages of mastitis
60	14	23.3	Mild
	7	11.7	Moderate
	1	1.7	Severe
Total	22	36.7	

The prevalence of mastitis in different breed viz; sahiwal cross, Friesian cross and local zebus are shown in table 5. Higher percentage of Friesian 50.0% cows are affected with mastitis compared with Sahiwal 32.0% and local zebu 26.7%. High yielding cross bred cows are prone to udder infection than low producing ones (Slettbakk el at, 1995; Radostits el at, 2000). The production of a large quantity of milk keeps the glandular tissue more generative and thus become more susceptible to infection. In this study, both sahiwal and Friesian crosses got higher rate than zebu. This result is consistent with the findings of Rahman (2004). Crossbred cows produce more milk than do zebu. Bigger size, long and pendulous udder in cross bred cows might have picked up more infection resting higher rate of infection (Roy et al, 1993)

Table 5 : Prevalence of mastitis in different breeds of cow

Types of breed	No. of cow examined	No. of affected	Prevalence (%)
Sahiwal Cross	25	8	32
Friesian Cross	20	10	50
Local Zebu	15	4	26.7
Total	60	22	36.7

The prevalence of mastitis in 3-4, 5-6, 7-8 years old cows were 33.3%, 36.8%, 40.0%. Prevalence of mastitis in this study varied depending on the age. Several studies were found in agreement with the present findings of increased mastitis in advance age (Slettbakk et al, 1995; Radostits et al, 2000Quaderi, 2005). Husain, (2007) showed that older cows with about 14 years of age had 61.0% sub clinical mastitis which correlates the present findings. Increased of age predispose the cow to more susceptible to infection and decreasing potency of the teat sphincter (pankey et al, 1991). On the contrary, Younger cows may have decreased susceptibility of mastitis because they possess more effective host defense mechanism (Dulin et al, 1988).

Table 6: Number and percentages of mastitis on the basis of age

Age (years)	No. of Cow examined	No. of infected	Prevalence (%)
3-4	12	4	33.3
5-6	38	14	36.8
7-8	10	4	40.0

The occurrence of mastitis in the cows of parity 1-2, 3-4, 5- above was 28.6%, 35.0% and 58.3% respectively . The prevalence of mastitis with lactation age differed significantly groups less than 0.01. Percentages of 5-above parity affected cows were higher. More

affection was observed with advancing of parity. A significantly better polymorphonuclear leukocyte function is active in primiparous cows than multiparous cows (Dulin et al. 1988). Interestingly, advancing parity cows are more productive, so it can be assumed that old aged and many parity cows are prone to mastitis occurrence (Table 7) .

Table 7 : Prevalence of mastitis in relation to parity

Parity	No. of cow examined	No. of Cow infected	Prevalence (%)
1-2	28	8	28.6
3-4	20	7	35.0
5- above	12	7	58.3

Prevalence of mastitis at lactation stages 1-2, 3-4, and 5-6 months were 33.3%, 38.1% and 40.0%, respectively. The prevalence of mastitis with lactation age differed significantly between groups. More percentages of cows of lactation stage 5-6 months had mastitis than that of 1-2 months lactation group. In this study, the prevalence of mastitis was higher in 5-6 months of lactation. This result is similar to other findings (Jha et al., 2010; Rahman, 2004; Kabir, 2003). The high rate of sub-clinical mastitis during the 6 months of lactation might be due to cow to cow transmission of contagious organism during milking.

Table 8: Prevalence of mastitis in different period of lactation

Period of lactation (month)	No. of cows examined	No. of cows infected	Prevalence (%)
1-2	24	8	33.3
3-4	21	8	38.1
5-6	15	6	40.0

The percentage of mastitis was 50%, 46.2%, 33.3%, 25.0% and 25.0% at milk yield of 1-5, 6-10, 11-15, 16-20 and 21-25 liters, respectively (Table 8). The percentage of prevalence decreases with higher milk production (Table 9). Significantly higher percentages of mastitis occurred in cows producing 1-10L of milk which was significantly higher compared with lactating producing 16-20 L or more milk

The prevalence of mastitis in this study was 50.0%, 46.0%, 33.0%, 25.0%, and 25.0% at milk yield of 1-5, 6-10, 11-15, 16-20 and 21-25 liters, respectively. This indicates that percentage

of prevalence decreases with higher milk production. This result is similar with other findings (Jha et al., 2010). The higher prevalence of mastitis in lower milk yielding cows is contradictory with various published results (Grohn et al., 1990; Chassagne et al., 1998; Rajala and Grohn, 1998; Grohn, 2000) studied the relationship between disease and milk production and stated that high milk yield predisposed a cow to certain diseases, particularly mastitis. Similarly Grohn et al. (1995) claimed that cows with mastitis produce more milk than do their healthy and generally lower yielding herd-mates. The sub- clinical mastitis may continue to reduce milk production and under certain stress condition (Pal and Verma, 1991). Clinical mastitis losses are generally apparent and consist of discarded milk, transient reduction in milk yield and premature culling (Fetrow, 2000). In this study, the dairy farmers of research area used to take less care to low yielding cows. Other explanation is that the chronic mastitis might have reduced milk yield in the cow. Because the milk yield was recorded on the day of examination which have resulted high mastitis in low yielding cows.

Table 9: Number and percentages of subclinical mastitis on the basis of milk yield

Milk yield (Liter)	No. of Cow examined	No. of infected	Prevalence (%)
1-5	12	6	50.0
6-10	13	6	46.2
11-15	15	5	33.3
16-20	12	3	25.0
21-25	8	2	25.0

A total of 60 milking cows were randomly selected for screening their quarter (n=240) subclinical mastitis by CMT. The proportion of different types of quarters affected with subclinical mastitis is shown (Table 10). Although the number of left rear quarter more affected than others. Among 240 quarter examined, only 11.7% (n= 28) of the quarters was found CMT positive. The percentage of CMT positive quarters was accordance with earlier reports (Husain, 2007). Shitandil et al., (2004) found that there was significant difference in the number of quarters affected in relation to their anatomical positions. Quarter wise testing

revealed the highest prevalence in the left rear quarters. Similar findings have been obtained by Jha et al, 2010; Singh and Baxi, 1980; Devi et al., 1997. In the present study, higher prevalence in this quarter could be due to more exposure to dung and urine. Moreover, pulling forwards and sideways may lead to undue stress on them while milking.

Table 10: SCM rate in number of position of quarter affected

Quarters	No. of quarters examined	Number of CMT positive quarters	Prevalence (%)
Right front	60	5	8.3
Left front	60	6	10.0
Right rear	60	8	13.3
Left rear	60	9	15.0
Total	240	28	11.7

The number of quarter affected in subclinical mastitis cows is shown in the diagram which shows that, single quarter affection were significantly higher.
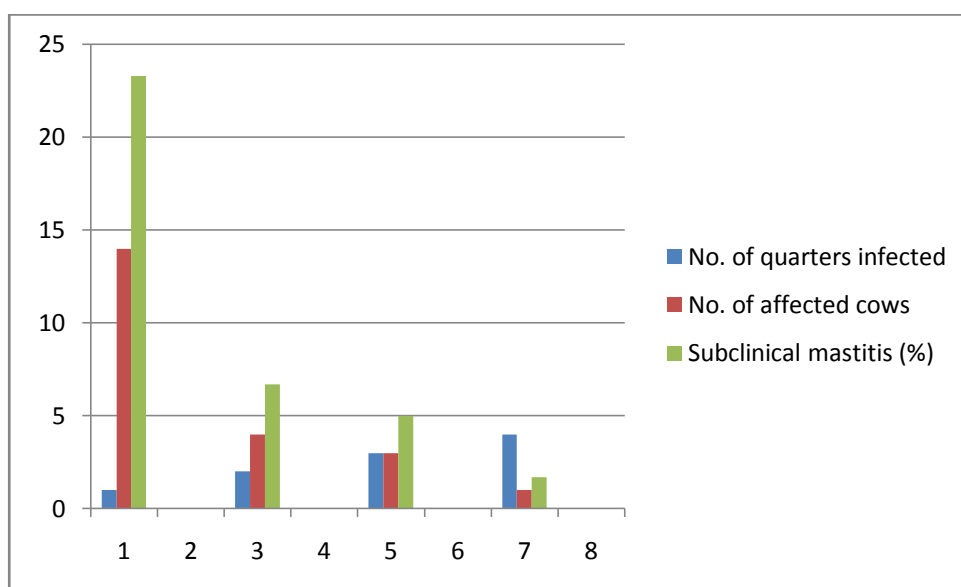

Figure 1: Prevalence of mastitis in relation to number of quarter infected
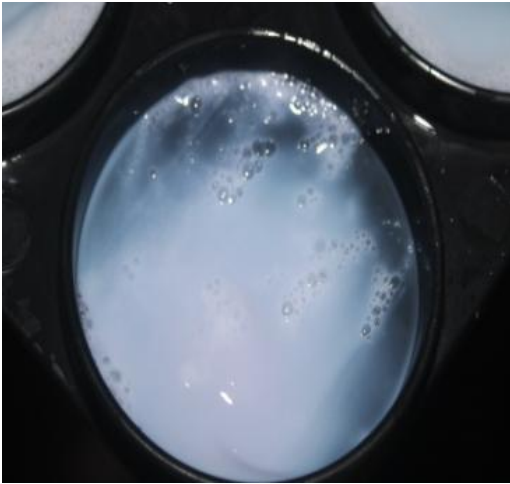
Figure 2: CTM Test,

Figure 3 : CTM Test,

Figure 4: CTM Test, Score

Chapter 4: Conclusions

The prevalence of subclinical mastitis in different breed was 36.7%. The current investigation showed that prevalence of the SCM among Sahiwal (25) and Friesian cross breed cows (20) higher than other breeds. SCM was diagnosed by using California Mastitis Test. Thus, there is need to improve managemental practices in the farms to decrease the prevalence of disease to a possible lower limit. Further, there is an urgent need to carry out a detailed epidemiological investigation to access the actual prevalence of SCM at national level.

Chapter 5: References

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Appendix

Questionnaire for data collection For Mastitis

Title :

Owner Name

....

Address ....

Occupation ....

Phone number ....

Total number of animal ....

Age ....

Breed ....

Clinical History ....

Clinical sign ....

Lactation period ....

Milk production ....

Diagnosis ....

Number of parity ....

Signature