PATHOLOGICAL INVESTIGATION OF MASTITIS IN DAIRY COWS AT BALIADANGI UPAZILA OF THAKURGAON DISTRICT

A Thesis
By

MD. AHSAN HABIB
REGISTRATION NO. 1605469

SESSION: 2016-2017

SEMESTER: JANUARY-JUNE, 2018

MASTER OF SCIENCE
IN
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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMADDANESH SCIENCEAND TECHNOLOGY UNIVERSITY, DINAJPUR

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Approved as to the style and content by

_____	_____
(Professor Dr. S.M. Harun-ur-Rashid)	**(Dr. Md. Golam Azam)**
Research Supervisor	Research Co-Supervisor

_____

(Professor Dr. S.M. Harun-ur-Rashid)
Chairman, Examination committee
and
Chairman

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

June, 2018

Dedicated
To My
Beloved Parents

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ABSTRACT

The present research was carried out by a cross-sectional study in lactating dairy cows at Baliadangi, Thakurgaon to determine the prevalence of subclinical mastitis (SCM) and to investigate the udder pathology of clinical mastitis (CM) during the period from January to June, 2018. To determine the prevalence, milk sample from a total of 280 dairy cows (245 crossbred and 35 local) were screened for subclinical mastitis using two indirect tests viz. California Mastitis Test (CMT) and Surf Field Mastitis Test (SFMT). Of all cows tested, 42.50% (n=119) and 41.42% (n=116) cows showed positive reaction for SCM by CMT and SFMT, respectively. The overall prevalence of SCM was 41.96% and CMT showed better performance in detecting SCM (37.58%) between the two indirect tests used. Higher prevalence of SCM was detected significantly ($P<0.05$) in milch crossbred cows (44.89%) in comparison to indigenous cows (25.71%). The prevalence gradually increased with advancing age where the prevalence of SCM was higher (47.05%) in age group more than 12 years than other age groups. The prevalence of SCM was significantly ($P<0.05$) highest in early lactation (50.41%) followed by mid (38.73%) and late lactation (31.25%). There was significant ($P<0.05$) association where the prevalence is higher (60.52%) with the increasing number of parity. High yielding cows showed higher prevalence and the prevalence of SCM was significantly ($p<0.05$) higher (66.66%) in cows yielding >10L of milk than others. Clinical inspection revealed discoloration of glandular tissue, blockage and nodule formation in teat canals, pus formation in the glands etc. Histopathological findings revealed significantly lower alveolar diameter, mass destruction of alveoli and udder parenchyma, reactive cell infiltration and fibrous tissue proliferation in mastitic dairy cattle. Results of this study may have application in selection of dairy animals and in a better understanding of the pathological consequences of mastitis.

Keywords: Prevalence, Subclinical mastitis, Udder, Histopathology, Dairy cow

CONTENTS

CHAPTER		PAGE
	ACKNOWLEDGEMENTS	i
	ABSTRACT	ii
	CONTENTS	iii-v
	LIST OF TABLES	vi
	LIST OF FIGURES	vii
I	**INTRODUCTION**	**1-2**
II	**REVIEW OF LITERATURE**	**3-11**
	2.1 Bovine Mastitis	3
	2.1.1 Overview on udder and its health	3
	2.1.2 Etiology	3
	2.1.3 Classes of Mastitis	5
	2.1.4 Pathogenesis of Mastitis	5
	2.1.5 Udder Defence Mechanism	6
	2.2 Prevalence of Subclinical Mastits	7
	2.3 Gross Examination of Udder of Mastitic Cows	9
	2.4 Histopathology of mammary gland of cows	10
III	**MATERIALS AND METHODS**	**12-21**
	3.1 Study Area and Period	12
	3.2 Husbandry and management system	12
	3.3 Survey Design and Sampling	12
	3.4 Questionnaire-based data collection	12
	3.5 Detection of Subclinical Mastitis by Indirect Tests	13
	3.5.1 California Mastitis Test (CMT)	13
	3.5.2 Surf Field Mastitis Test (SFMT)	14
	3.6 Data analysis	15
	3.7 Clinical inspection of udder	15
	3.8 Histopathological Study	15

CONTENTS (Contd.)

CHAPTER		PAGE
IV	**RESULTS**	**22-30**
	4.1 Overall Prevalence of Subclinical Mastitis by Indirect Tests	22
	4.1.1 Prevalence on the basis of indirect tests	22
	4.1.1.1 Breed wise prevalence	22
	4.1.1.2 Age wise prevalence	23
	4.1.1.3 Lactation stage wise prevalence	24
	4.1.1.4 Parity Related Prevalence	25
	4.1.1.5 Milk Yield Related Prevalence	26
	4.2 Necropsy Findings of Clinical Mastitis	27
	4.3 Histopathological Findings of Clinical Mastitis	29
V	**DISCUSSION**	**31-36**
	5.1 Overall Prevalence of Subclinical Mastitis	31
	5.1.1 Breed wise prevalence	31
	5.1.2 Age wise prevalence	32
	5.1.3 Lactation stage wise prevalence	33
	5.1.4 Parity Related Prevalence	33
	5.1.5 Milk Yield Related Prevalence	34
	5.2 Necropsy Findings	34
	5.3 Histopathological Investigation	35
VI	**CONCLUSIONS**	**37**
	REFERENCES	**38-44**

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Scoring of California mastitis test results	13
2	Prevalence of subclinical mastitis by indirect tests	22
3	Breed wise prevalence of SCM in dairy cows	23
4	Age wise prevalence of SCM in dairy cows	23
5	Prevalence of SCM on the basis of lactation stage	24
6	Parity related prevalence of SCM in dairy cows	25
7	Prevalence of SCM related to milk yield	26

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE NO.
1	Breed wise prevalence of mastitis in dairy cows	23
2	Age wise prevalence of mastitis in dairy cows	24
3	Lactation stage wise prevalence of mastitis in dairy cows	25
4	Parity related prevalence of mastitis in dairy cows	26
5	Milk yield related prevalence of mastitis in dairy cows	27

LIST OF PHOTOS

PHOTO NO.	TITLE	PAGE NO.
1	CMT and SFMT Test Kits	19
2	CMT result interpretation	19
3	SFMT Test result interpretation	19
4	Inspection and palpation of affected udder	20
5	Presence of blood in milk	20
6	Abnormal distance between the two hind quarters and laceration on one of the teat	20
7	Hyperkeratosis and blindness of tip of teat and total loss of one teat	20
8	Presence of nodules on the teats	21
9	Swollen udder with pus formation	21
10	Tissue preparation protocol for histopathology	21
11	Sectioning of tissue block by microtome machine	21
12	Microscopic examination of prepared slides	21
13	Cut section of a mastitic udder	28
14	Pus accumulation in the streak canal along with abnormal tissue growth	28
15	Haemorrhagic mucosa of teat canal	28
16	Nodule formation at the base of teat canal	28
17	Pus accumulation in the gland	29
18	Cut section of glandular tissue with extensive nodular formation	29
19	Coagulated protein precipitates in the milk alveoli	30
20	Mass destruction of alveoli along with fibrous tissue proliferation	30
21	Mass destruction of udder parenchyma with alveolar atrophy	30
22	Huge infiltration of reactive cells	30

CHAPTER I
INTRODUCTION

An increase in the global population coupled with the increasing demands for milk as an economic food and as an industrial raw food product has necessitated an increase in production by dairy farms (Javaid *et al.,* 2009). Milk quantity, quality and production efficiency of cows is directly dependent on the udder health (Szencziová *et al.,* 2013). The udder is the most important part of the body of the dairy cow and its morphological and physiological characteristics affect health of cows and play a vital role in sustainable economic milk production (Gulyas and Ivancsics, 2002; Tilki *et al.,* 2005; Tancin *et al.,* 2007). It is also recognized that the udder characteristics are very important in respect to milk production (Shukla *et al.,* 1997).

Bovine mastitis is a single most common disease syndrome of adult dairy cows recognized mainly as clinical and sub-clinical types worldwide, which has great economic impact on dairy industry with complex multifactorial etiology (Nooruddin *et al.,* 1997). It is estimated that one third of all dairy cows are infected with some form of mastitis in one or more quarters. It can occur in clinical and subclinical form. Clinical mastitis is readily apparent and easily detected by abnormalities in milk or the udder or the occurrence of secondary clinical signs. Subclinical mastitis does not lead to visible changes in milk or udder. Sub-clinical mastitis may cause heavy economic losses due to reduced milk production, discarded milk, early replacement of animal, reduced sale value and costly veterinary treatment (Singh *et al.,* 1994; Samad and Awaz, 1997).

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, subclinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk *et al.,* 2003). Cows with subclinical mastitis are those with no visible changes in the appearance of the milk and/or the udder, but milk production decreases by 10 to 20% with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing (Holdway, 1992). Although there are no visible or palpable external changes, the infection and inflammation is occurs in the udder (Blowey and Edmondson, 1995).

The Clinical Mastitis (CM) is accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). Histopathological

findings clinical mastitis reveal significantly lower alveolar diameter, number of alveoli and alveolar epithelial cell population in mastitic dairy cattle. The Sub clinical mastitis (SCM) can be known only after laboratory examination, as there are no gross inflammatory changes in the udder tissue. In almost all cows microorganisms are mainly 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. Predisposing factors such as poor management and hygiene, teat injuries and faulty milking machines are known to hasten the entry of infectious agents and the course of the disease (Majic *et al.*, 1993).

The CM can easily be detected by inspection of udder and or systemic signs of inflammation, whereas, diagnosis of subclinical mastitis is more problematic since the milk appears normal. Various methods, based on physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of subclinical mastitis (Emanuelson *et al.*, 1987). 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. Therefore, indirect tests viz. California Mastitis Test (CMT), White Side Test (WST), Surf Field Mastitis Test (SFMT) etc. can be used which are simple and do not require any complex laboratory equipment. Reagent of these tests is solutions containing detergents. Detergents decrease surface tension, change the structure and conductivity of cell membrane and nucleus, disturb osmotic balance, block oxidizing and stimulate proteolytic enzymes, and increase milk viscosity (Middleton *et al.*, 2004).

Baliadangi upazila of Thakurgaon district is a traditional milk producing area. Information on prevalence of bovine mastitis and their associated risk factors in this area is rare. Therefore, it was crucial to carry out pathological investigation of mastitis of dairy cows in this area.

The research was undertaken with the view of the following specific objectives:

i) To determine the prevalence of subclinical mastitis (SCM) in dairy cows.

ii) To inspect the udder morphology and necropsy findings and to investigate the histopathological changes of mammary tissue affected with clinical mastitis (CM).

CHAPTER II
REVIEW OF LITERATURE

2.1 Bovine Mastitis

2.1.1 Overview on udder and its health

Udder health has been an important component of dairy farming for the last decades. Udder health disorders were always related to a decreased profitability, and an increase in unexpected culling. More recently, udder health is becoming more important due to strict milk quality regulations. This has further increased the attention of the dairy industry towards udder health. Udder health is affected by a number of interrelating components such as presence and pathogenicity of microorganisms, environment and management, cow factors such as conformation and immunological performance, and treatment and prevention strategies (Poso and Mantysaari, 1996).

In order to understand mastitis pathobiology, the mammary gland has been studied with respect to its anatomy, physiology, and the genomics (Ogorevc *et al.,* 2009). The interior of each quarter is composed of a teat cistern, gland cistern, milk ducts and glandular tissue. The secretary portion known as the glandular tissue contains millions of microscopic sacs called alveoli. Each alveolus is lined with milk- producing epithelial cells and is surrounded by muscle cells which contract and squeeze milk from the alveolus during milking. Blood vessels bring nutrients to each alveolus where the epithelial cells convert them into milk. Milk accumulates in the alveolar spaces, milk ducts and cisterns between milking. It is through the teat duct that the accumulated fluid is removed during milking (Seffner and Pfutzner, 1980).

2.1.2 Etiology

Mastitis can be cause by many pathogens. It can be classified as either major or minor pathogens. The major pathogens can be further subdivided into contagious and environmental infection agents (Radostits *et al.,* 1994). Infection cause by contagious pathogens is transmitted directly from cow to cow. The most common contagious organisms are *Staphylococcus aureus, Streptococcus agalactiae, Corynebacteriumbovis and Mycoplasma* species. Contagious microorganisms are well adapted to survival in the

udder and usually establish mild clinical infections of long duration as chronic infections (Erskine, 2001).

The main environmental organisms are gram-negative bacteria. The gram-negative bacteria there are *Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Seratia, Pseudomonas species, Proteus and Actinomyces pyogenes.* The environmental *Streptococci* include *Streptococcus uberis, Streptococcus dysgalactiae, and Streptococcus equinus.* Infections causedby environmental pathogens are frequent and of short duration. These infections usually result in clinical mastitis (Smith and Hogan, 1993).

Fox and Gay (1993) studied that contagious mastitis caused by major pathogens are *Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium bovis, Mycoplasma sp*, and *Streptococcus dysgalactiae*. These pathogens are discussed relative to prevalence, virulence factors, pathology, and control. These control measures include milking time hygiene, segregation, culling, vaccination, and treatment.

Smith and Hogan (1993) studied that Environmental mastitis affects all dairy farms and generally is the major mastitis problem on modern, well managed dairy farms. Control measures effective against contagious pathogens are of little value in controlling of environmental pathogens. Control of environmental mastitis is achieved by reducing exposure of teat ends to environmental pathogens and by maximizing the resistance of the cow to intramammary infection. Significant sources of environmental pathogens are organic bedding materials, manure covered alleyways, and wet or damp areas in barns, exercise lots, or pastures. Milking time hygiene can influence teat-end exposure. In general, exposure is minimized when all areas of the environment are clean, cool, and dry. Resistance is maximized by providing a stress-free environment that minimizes teat-end injury, and by feeding balanced diets sufficient in vitamin E and selenium. Antibiotic therapy during lactation or the dry period is of little value in the control of environmental mastitis in dairy herds, with the exception of preventing environmental streptococcal infection during the early dry period. Effective vaccines may help reduce the impact of environmental mastitis in the near future.

2.1.3 Classes of Mastitis

It is subdivided into clinical mastitis (inflammation with visual signs of inflammation in the udder or milk) and subclinical mastitis (inflammation without visual signs). Both clinical mastitis and subclinical mastitis influence milk quality and yield negatively, and mastitis is therefore of major economic concern for the farmer. Clinical mastitis is also of potential concern from an animal welfare perspective (Lundberg, 2015).

Clinical mastitis is defined as an infection of the udder that results in visible changes in the udder quarter and milk (Rodenburg, 1990), may it be acute, sub-acute or chronic. The development of clinical mastitis in dairy cows can be detected with high sensitivity and specificity in advance of visible changes in foremilk or udder tissue by determining the electrical conductivity of the foremilk (Milner *et al.,* 1997). Clinical mastitis is characterized by visual clots or discolorations of the milk, often in combination with tender and swollen udder, sometimes in combination with fever, loss of appetite *etc* (Bengtsson *et al.*, 2005).

Guidry (2007) stated that subclinical mastitis is characterized by changes in milk composition (*e.g.* somatic cell count, leukocytes and epithelial cells), and changes in milk pH and ion concentration, without clinical signs of inflammation. In the healthy lactating mammary gland, the milk SCC is often <100,000 cells/mL of milk, while the SCC can increase to >1,000,000 cells/ml of milk during subclinical mastitis. The major factor affecting the SCC at the herd and individual level is the presence of intramammary infections (IMI).

2.1.4 Pathogenesis of Mastitis

Intramammary infection results once bacteria pass through the teat duct of a mammary quarter, multiply in the teat and gland cisterns, and progress dorsally to the milk-producing tissues (Akers and Nickerson, 2011). After entry through the teat canal the bacteria enter into the udder tissue, multiply and produce toxins causing inflammation of the udder or the corresponding teat. Due to inflammation, the body releases leucocytes and the quality of the milk gets affected. The milk becomes watery or curdled; sometimes blood streaks may also be present depending on the severity of infection. Infection of the udder usually takes place directly through teat canal. But, organisms may get settled in the mammary tissues via blood in case of tuberculous mastitis. Broadly,

two stages have been described *viz.* invasive stage and infective stage. In invasive stage the organism gets entry from the exterior to the teat canal and milk. Infective stage denotes the stage of bacterial multiplication and their resultant damaging effect on the mammary tissues (Sol *et al.,* 2000).

Once the organisms breach the teat duct and the cisternal spaces of the udder, adherence of bacteria to tissues lining the interior of the mammary gland may affect their ability to remain inside the gland, especially during lactation when the contents of the udder are periodically flushed during each milking; up to 4 times a day, or more with robotic milking. Interaction of bacteria with milk leukocytes affects the establishment of infection. In milk of uninfected, healthy mammary glands, macrophages are the predominant leukocyte type, and serve as nonspecific sentinels for the detection of invading pathogens (Sol *et al.,* 2000; Akers and Nickerson, 2011).

After detection of bacteria, macrophages release chemoattractants that recruit polymorphonuclear neutrophilic leukocytes from the vasculature into the area of infection. The polymorphonuclear neutrophilic leukocytes extravasate in large numbers, and initially accumulate around alveoli, with the goal of migrating across the alveolar, ductal, and cistrnallumina to contact, engulf, and kill the invading pathogens. Inflammation that ensues in response to bacterial presence is initiated by the release of interferons, interleukins, and tumor necrosis factor (TNF-α) (Akers and Nickerson, 2011).

2.1.5 Udder Defence Mechanism

The antibacterial defence of bovine udder comprises of anatomical features of the gland and humoral and cellular defence mechanisms (Colditz and Watson, 1982) that can be separated into two distinct categories: innate immunity and specific immunity. The optimal protection is the collaborated function of the both above mentioned immune mechanism. Innate immunity, also known as nonspecific responsiveness, is the predominant defence during the early stages of infection. Nonspecific responses are present or are activated quickly at the site of infection by numerous stimuli; however, they are not augmented by repeated exposure to the same insult. Nonspecific or innate responses of the mammary gland are mediated by the physical barrier of the teat end,

macrophages, neutrophils, natural killer cells, and by certain soluble factors (Rainard and Riollet, 2006).

The specific or acquired mammary immune system recognizes specific determinants of a pathogen. Activation of specific mammary immune defences results in the selective elimination of mastitis-causing pathogens. Recognition of pathogenic factors is mediated by several lymphoid populations, macrophages, and antibody molecules. Because of the "memory" of certain lymphocytes, specific immune responses can be augmented by repeated exposure to a pathogen. Vaccination of dairy cattle against certain pathogens can occur if specific mammary immune mechanisms are effectively activated (Sordillo and Streicher, 2002).

Keratin present within teat duct is one of the most important limiting factors in penetration of bacteria via teat duct which is major entry site for most of mammary pathogens. The infectious agents that are not blocked by keratin and get entry into gland are attacked by humoral and cellular defences. The humoral defense comprises of different antimicrobial molecules and enzymatic pathways while cellular defense consists of different types of defense cells including lymphocytes, macrophages and neutrophils (Oviedo-Boyso *et al.,* 2007)

2.2 Prevalence of Subclinical Mastits

Barua (2014) studied A total of 444 quarter samples of 111 (56 from commercial dairy farms and 55 from backyards) lactating dairy cows were considered. Sub-clinical mastitis (SCM) was determined using three different indirect screening tests: California Mastitis Test (CMT), White Slide Test (WST) and Surf Field Mastitis Test (SFMT). Sensitivity and specificity were also determined to measure the accuracy of those tests. The prevalence of SCM by CMT, WST and SFMT were 32.43% (n=144), 33.56% (n=149) and 31.53% (n=140), respectively. Distribution of SCM in relation to different variables at quarter level and animal level was also recorded. The prevalence of SCM was significantly ($P<0.05$) higher in aged, high yielding cows in addition with history of periparturient diseases, without dry cow therapy both at quarter and animal level. A significantly ($p<0.01$) higher prevalence (48.98%) of SCM was observed in higher parity number (>4) than others at quarter level. No significant difference ($P>0.05$) was found in relation to breed. Using CMT as a gold standard, sensitivity and specificity of WST and

SFMT were also calculated at 95% confidence interval. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, negative predictive value and disease prevalence by WST and SFMT were comparable.

Islam *et al.* (2011) carried out a research work to determine the prevalence of subclinical mastitis in lactating Dairy Cow of Bangladesh Agricultural University dairy farm (BAUDF) and rural areas of Tangail sadar upazila of Bangladesh during the period of July 2009 to April 2010. A total of 200 milk samples (40 from BAUDF and 160 from Tangail sadar upazila) were collected for this study which were subjected to physical examination and subsequently screened for subclinical mastitis using three indirect tests viz. White Side Test (WST), California Mastitis Test (CMT), and Surf Field Mastitis Test (SFMT). Overall prevalence of subclinical mastitis (SCM) in lactating dairy cows found in this study was 29%. Cows were infected with SCM 29.5%, 27.5% and 25.5% detection by CMT, WST and SFMT respectively. Higher prevalence of SCM was detected in milch crossbred cows (36.36%) in comparison to local bred cows (24.61%) maintained under extensive management system in Rural area of Tangail sadar upazila. The prevalence of SCM was recorded in 31.58%, 30.76% and 68.75% in cows of local area of Tangail sadar upazila, and 25.0%, 40.0% and 71.42% in cows of BAU, DF during the early, mid and late stages of lactation respectively. The highest prevalence of SCM was recorded during the early lactation stage in both the local breed cows (30.0%) and cows of BAUDF (45.83%) in comparison to their respective mid and late stages of lactation. The prevalence of SCM was highest in lactating cows having third lactation and high yielding (cows produced >10 liter milk per day) both in local breed and crossbred cows.

Islam *et al.* (2010) also studied a total of 330 lactating dairy cows at Baghabari, Sirajganj to determine the prevalence and risk factors of clinical (CM) and sub-clinical (SCM) mastitis using California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT) during the period from July to December, 2009. Of all cows tested, 2.12% (n=7) cows were affected with CM and 37.58% (n=124), 36.67% (n=121) and 35.15% (n=116) cows showed positive reaction for SCM by CMT, WST and SFMT respectively. The overall prevalence of SCM was 36.46% and CMT showed better performance in detecting SCM (37.58%) among three indirect tests used. The prevalence of SCM was significantly ($p<0.01$) higher (47.61%) in age group more than 13 years than others. A significantly ($p<0.01$) higher prevalence of SCM was observed in parity

number more than 11 than others. The prevalence of SCM was significantly ($p<0.01$) higher (37.12%) in cows yielding >10L of milk than others. The prevalence of SCM was highest in late lactation (72.45%) followed by early (40%) and mid lactation (27.56%). Herds having 16 or more milch cows had significantly ($p<0.05$) higher SCM than those with fewer milch cows.

Rahman *et al.* (2009) observed the prevalence of mild mastitis was 17.3% and 40.7%, whereas that of moderate mastitis was 2.6% and 4.1 % in dry and wet seasons, respectively. Some selected physiological and managemental factors were studied by Bilal *et al.* (2004) to determine the effect of clinical mastitis in buffalo. He showed that the prevalence of clinical mastitis was higher in peri-urban (25.21%) than rural (19.74%) areas. The highest incidence was observed during 4 to 6 months after calving both in peri-urban (45.67%) and rural (45.08%) areas. The maximum cases of mastitis were found during third lactation both in peri-urban (19.00%) and rural (22.98%) areas.

2.3 Gross Examination of Udder of Mastitic Cows

Klaas *et al*. (2004) stated that the systematic clinical examinations of udders are an additional tool for the evaluation of udder health status on dairy farms. Houe *et al.* (2002) stated evaluation of pathological parameters such as nodes in the udder, skin lesions and oedema showed good agreement between clinicians for the determination of udder health. The "mastitis udder" was characterized by the clinical variables asymmetry between hind quarters, knotty tissue, and acute clinical mastitis, reduced milk yield and high SCC were related to the "mastitis udder," whereas low SSC was related to the "small udder" (Klaas *et al*., 2004). Sharma and Singh (2006) found teat lesions were associated with 23.58% clinical cases of mastitis. Shukla *et al*. (2005) examined udder for injury, pain, swelling, hardness and fibrosis. Teat shape was categorized into funnel, round, flat and plate types, while on the basis of length, teats were classified as small (<5.5 cm), medium (5.5-7.5 cm) and large (>7.5 cm).

Hussain *et al.* (2010) studied mammary glands of cows for presence of any gross lesions. The udder configuration and teat/udder pathology was recorded following Bhutto *et al*. (2010). Teat and streak canal length and teat diameter from apex, mid and base of all the teats were measured using a vernier caliper after the animals were slaughtered. The teat and udder shape was documented following Shukla *et al.* (1997). The results revealed lower ($P<0.0001$) teat and teat canal length; whereas, higher ($P<0.0001$) teat base and

mid diameter were recorded in mastitic cattle. Morphometric comparisons revealed lower (P<0.0001) teat and teat canal length; whereas, higher (P<0.0001) teat base and mid diameter in mastitic cattle. The results of univariate and bivariate logistic regression including species and individual variable in the model revealed association of teat involved, teat shape, udder shape, somatic cell count and teat/udder pathology with the occurrence of mastitis.

2.4 Histopathology of mammary gland of cows

Hussain *et al.* (2012) reported that histopathologically, the udder section from healthy cattle showed no pathological lesions and the milk secretion was observed in alveoli. However, the tissue sections from mastitic animals revealed mild, moderate or severe atrophy of alveoli. The cellular exudate in udder tissue was present in the lumen of the alveoli in varying amounts in a number of cases. The acute inflammatory changes were recorded in 56% and chronic inflammation in 44% cattle. Infected mammary parenchymal tissues showed destruction of alveoli and fibrous tissue proliferation. Cellular infiltration mainly was observed in different areas of mammary tissues such as in teat cistern lining, gland cistern and deep parenchyma. Mastitic udder showed significantly lower alveolar epithelial cell population, alveolar luminal diameter and number of alveoli per plate.

The study done by Benites *et al.* (2002) evidenced for repair process replaces permanently the glandular tissue by connective tissue and consequently leads to reduction in milk production. In this study, it was observed that 71 (55.5%) of the samples which had positive microbiological results, showed inflammatory response associated to repair and 10 (7.8%) samples showed only repair process. Therefore a total of 81 (63.3%) of these samples showed loss of parenchyma, the area where milk is produced, due to the repair process (fibrosis and/or cystic dilatation).

Histological analyses have been widely used since the 1970s and are still being used today for assessing damage to secretory tissue in the bovine mammary gland caused by mastitis pathogens. Benites *et al.* (2002) revealed 96.9% (n=184) of samples showed inflammatory response. According to Zhao and Lacasse (2007) lesions of the breast tissue reduces the number and activity of epithelial cells and therefore contributes to lower milk production with increasing proportions of lymphocytes and macrophages and a decrease cell number of polymorphonuclear cells.

According Trinidad *et al.* (1990), mammary glands infected with *Staphylococcus aureus,* showing an increase of connective tissue inter-cellular and a reduction in epithelial cells and alveolar lumen. Kheira and Abdellatif (2014) found 34.61% of samples showed inflammatory lesions different from the disappearance of the alveolar lumen, through fibrosis to the complete destruction of the parenchyma. When the infection persists and the channels are blocked, the milk within the alveoli increases the pressure there, the secretary cells lose their ability to synthesize and the cells begin to atrophy. Substances released by white blood cells cause destruction of cellular structures, which are replaced by scar.

CHAPTER III
MATERIALS AND METHODS

3.1 Study Area and Period

A cross-sectional study was carried out on a total of 280 lactating cows (245 crossbred and 35 indigenous) at Baliadangi, Thakurgaon to determine the prevalence of subclinical mastitis using California Mastitis Test (CMT) and Surf Field Mastitis Test (SFMT) during January to June, 2018. Histopathological research works were done at research laboratory of Department of Pathology and Parasitology at Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.2 Husbandry and management system

Crossbred and indigenous dairy cows at rural areas of Baliadangi upazila reared under both semi intensive and extensive husbandry system with ground muddy floor were taken under the study. They were provided with green grass in addition to natural pasture with concentrated feed as well.

3.3 Survey Design and Sampling

A cross-sectional study was undertaken using random sampling technique. 104 households and 13 small scale dairy farms were randomly selected. Almost all lactating cows of the selected herds were included to take milk samples. Sample size (280) was determined following the procedure described by Putt *et al*. (1987) assuming an expected prevalence of 30%.

3.4 Questionnaire-based data collection

A pre-tested questionnaire was used to collect both herd and animal level data including type of dairy husbandry system, breed, age, parity, lactation stage, milk yield and herd size. Udder and milk abnormalities (injuries, blindness, tick infestation and indurations, swelling, milk clots, abnormal secretion, etc.) were recorded. Depending on the clinical inspection findings, cases were categorized as clinical mastitis positive or negative. Age of the animals was determined by asking the owner and dentition characteristics and categorized as 2-3, >3-5, >5-8, >8-12, >12 years and above. Stage of lactation was categorized as early (1^{st} to 3^{rd} month), mid (4^{th} to 6^{th} month), and late (above 6^{th} month to the beginning of dry period). Parity was categorized as 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and above.

3.5 Detection of Subclinical Mastitis by Indirect Tests

Sub-clinical mastitis was diagnosed based on results of indirect tests and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively. The following indirect tests were used for detection of subclinical mastitis in dairy cows.

3.5.1 California Mastitis Test (CMT)

The CMT kit (Leucocytest®, Synbiotic Corporation, France) was used for screening of milk samples for subclinical mastitis. The procedure of CMT was performed as per manufacturer's instruction, in brief; 2 ml milk was drawn into the cup and an estimated equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored. The CMT results were read immediately as per manufacturer's recommendation and were scored based on the amount and thickness of gel formed as described by Ikram (1997) as shown in Table 1.

Table 1. Scoring of California mastitis test results

Reading			**Interpretation**	
Aspect	**Score**		**Infection**	**Related with the average cellular NUMERATION (x 10^3/ml)**
	Value	**Cross**		
Consistency normal or gray color.	0	(0)	Absent	100
Light gel disappearing after stirring or Purplish gray color	1	(±)	Infection risk by minor pathogenic	300
Light persistent gel-crumbly filaments or Purple gray	2	(+)	Sub-clinical mastitis	900
Immediate thickening viscous cluster at the bottom of the well.	3	(++)	Sub- clinical mastitis	2700
Thick gel, egg white consistency with dark purple color.	4	(——)	Sub-clinical mastitis near the clinical expression	8100

3.5.2 Surf Field Mastitis Test (SFMT)

This test was performed and scored following the method described by (Muhammad *et. al.,* 1995). In brief, 2 ml milk was drawn into the cup and 2 ml reagent (4% solution of Surf Excel®, Uniliver, Bangladesh) was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored as 1+, 2+, 3+ and 4+.

The samples were subjected to Surf Field Mastitis test (SFMT). The principle of the test is that when detergent is added into milk sample, it causes rupture of somatic cell and release DNA and other cell contents. DNA is acid in nature, while detergent contains alkyl-arylsulfonate, which is basic in nature. DNA and detergents unite to form a gel; consistency of gel depends upon the number of somatic cells. More cells more thick gel and vice versa.

Procedure

1. 3% Surf solution (pH = 10.3): 3 g of commonly used surf (detergent) powder (Surf Excell, Uniliver, Bangladesh) was dissolved in 100 ml of clean tap water. The test solution is stable for 6 month at room temperature.

2. A plastic paddle with four receptacles for the respective quarters of an animal was used to draw the milk sample.
3. Equal quantity of 3% reagent and milk was taken in the paddle or container. The mixture was swirled for about 1 minute and then examined visually for the presence of small floccules and gel.

The change in consistency of milk indicated mastitis, while no change in consistency of milk indicated healthy samples. The mastitis was graded into further four categories based on the severity of disease from lower to higher intensity as, + = moderate, + + = severe, + + + = more severe, + + + + = very severe (Muhammad *et al.,* 1995). The percentage of prevalence was calculated.

The prevalence was expressed in percent positive by using the following formula:

Prevalence (%) = No. of positive tests/Total no. of tests×100

3.6 Data Analysis

All collected data were entered into Microsoft Excel spreadsheet. The prevalence of clinical mastitis was the dependent variable while breed, age, parity, lactation stage, and milk yield and quarter involvement were independent variables considered at cow level. The independent variables at herd level include barn floor status and hygienic strategy. The association between dependent and independent variables were tasted by logistic regression. For analysis of data SPSS version 20 was used.

3.7 Clinical Inspection of Udder

The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, atrophy of the tissue and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Appearances of milk from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. Injuries caused by vigorous calf suckling were identified as circumscribed lesions around the teats. Immediately after collection, milk samples were subjected to physical examination with naked eyes to detect any abnormalities in colour, odour, consistency and presence of clot, blood, flakes and any other visible abnormalities.

3.8 Histopathological Study

During necropsy, udder was collected, preserved in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin according to standard method (Luna, 1968). Details of tissue processing, sectioning and staining are given below.

3.8.1 Equipment and Appliances:

4. Samples (Udder of cattle)
5. 10% neutral buffered formalin
6. Chloroform
7. Paraffin
8. Alcohol
9. Tape water

10. Xylene
11. Hematoxylin and Eosin stain
12. Distilled water
13. Clean slides
14. Cover slips
15. Mounting media (DPX)
16. Microscope

3.8.2 Processing of Tissues and Sectioning

- The tissues were properly trimmed into a thin section to obtain a good section of the tissue.
- The tissues were washed under running tap water for overnight to remove the fixative.
- The tissues were dehydrated in ascending grades of alcohol to prevent shrinkage of cells using 50%, 70%, 80%, 90% alcohol and three changes in absolute alcohol, for 1hr in each.
- The tissues were cleaned in two changes in chloroform to remove alcohol, 1.5 hr in each.
- The tissues were embedded in molted paraffin wax at 56-60^{0}C for two changes, 1.5 hr in each.
- Paraffin blocks containing tissue pieces were made using templates and molted paraffin.
- Then the tissues were sectioned with a microtome at 5-6µm thickness. The sections were allowed to spread on luke warm water bath (40-45°C) and taken on a glass slide. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides containing sections were air dried and stored in cool place until staining.

3.8.3. Routine Hematoxylin and Eosin Staining Procedure

Preparation of Harris' Hematoxylin Solution

Hematoxylin crystals	5.0g
Alcohol (100%)	50.0 ml
Ammonium or potassium alum	100 g
Distilled water	1000.0 ml
Mercuric oxide (red)	2.5 g

Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

Preparation of Eosin Solution

1% Stock Alcoholic Eosin

Eosin Y, water soluble	1 g
Distilled water	20 ml
95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added**.**

Working Eosin Solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

Staining Protocol

The sectioned tissues were stained as described below:

- Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 minutes in each).
- Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 minutes in each; 95% alcohol for 2 minutes; 80% alcohol for 2 minutes; 70% alcohol for 2 minutes) and distilled water for 5 minutes.
- The tissues were stained with Harris' hematoxylin for 10 minutes.
- The sections were washed in running tap water for 10-15 minutes.
- Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol), 2-4 dips.
- The tissue sections were then washed in tap water for 5 minutes and dipped in ammonia water (2-4 times) until sections became bright blue.
- The sections were stained with eosin for 1 minute and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 minutes in each).
- The stained sections were then cleaned by 3 changes in xylene, 5 minutes in each and finally the sections were mounted with cover slip using DPX.
- The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

Photo 1. CMT and SFMT Test Kits

Photo 2. CMT result interpretation

Photo 3. SFMT Test result interpretation

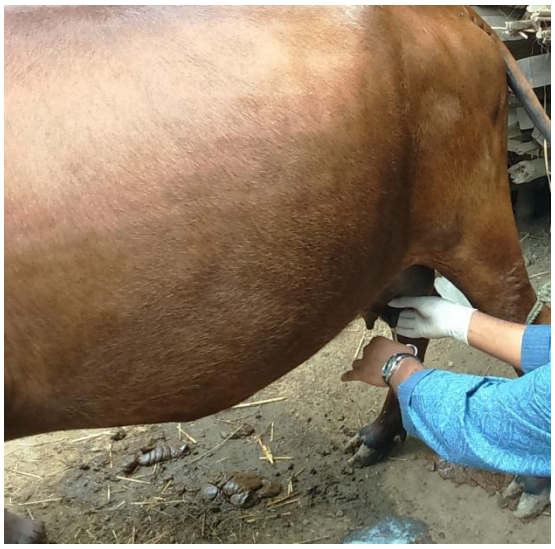

Photo 4. Inspection and palpation of affected udder

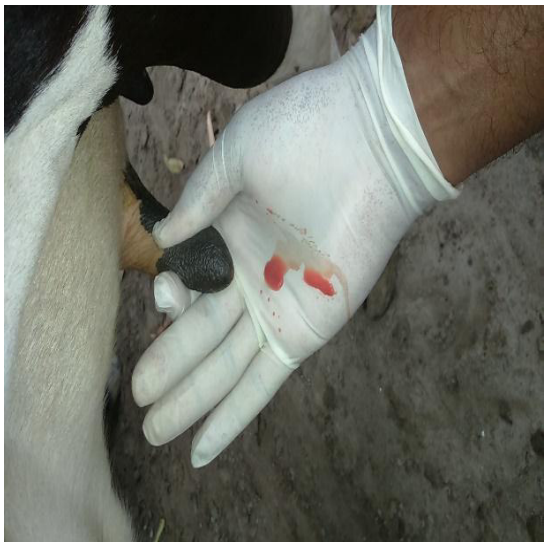

Photo 5. Presence of blood in milk

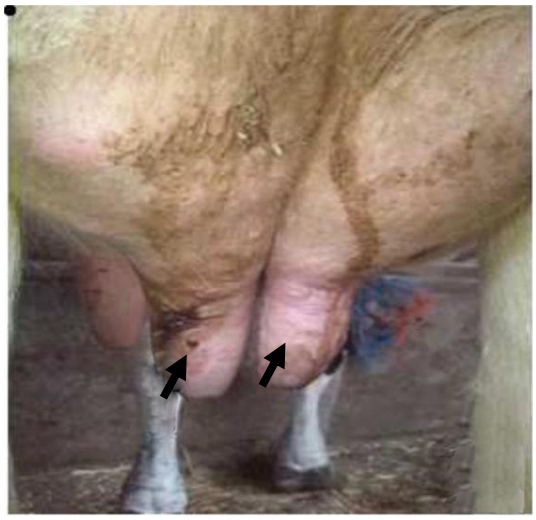

Photo 6. Abnormal distance between the two hind quarters and laceration on one of the teat

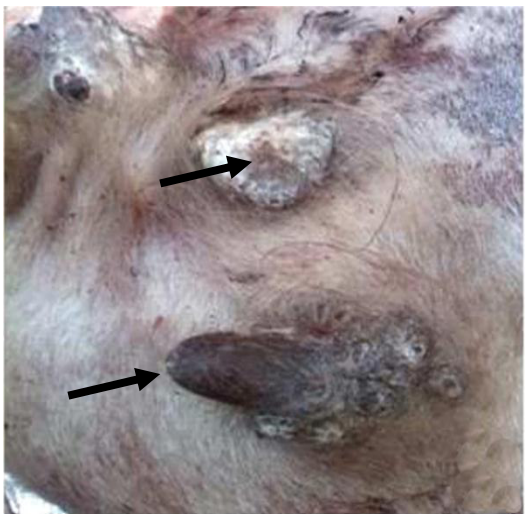

Photo 7. Hyperkeratosis and blindness of tip of teat and total loss of one teat

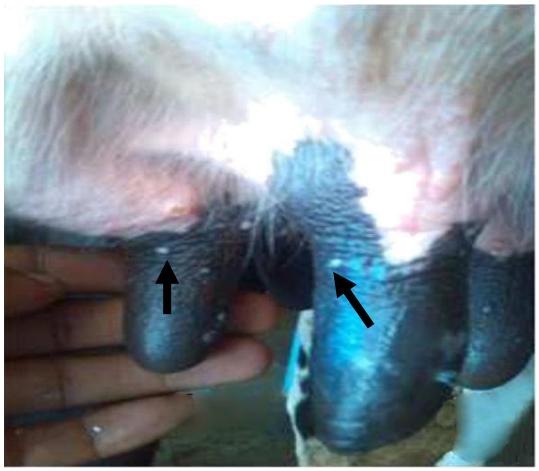

Photo 8. Presence of nodules on the teats

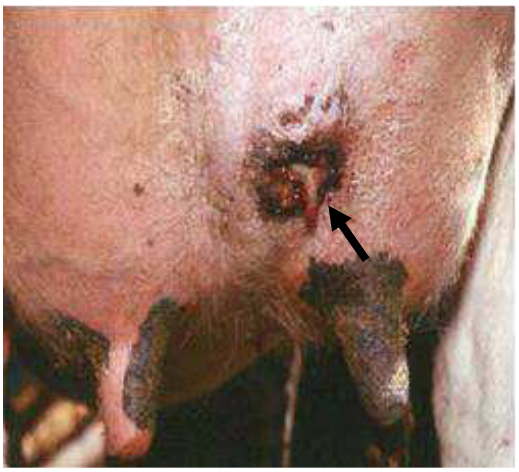

Photo 9. Swollen udder with pus formation

Photo 10. Tissue preparation protocol for histopathology

Photo 11. Sectioning of tissue block by microtome machine

Photo 12. Microscopic examination of prepared slides

CHAPTER IV
RESULTS

4.1 Overall Prevalence of Subclinical Mastitis by Indirect Tests

The study was conducted on mastitis in cows at Baliadangi upazila of Thakurgaon district for a period of six months starting from 1st January to 31th June, 2018. It was carried out to determine the overall prevalence of subclinical mastitis in cows by indirect tests viz. california mastitis test (CMT) and surf field mastitis test (SFMT) in relation to observe breed, age, lactation, parity, milk yield and udder quarters.

4.1.1 Prevalence on the basis of indirect tests

The prevalence of subclinical mastitis was recorded 42.50% (CMT) and 41.42% (SFMT) where CMT showed higher prevalence than SFMT. Overall prevalence of subclincal mastitis by indirect tests was 41.96% (Table 2).

Table 2. Prevalence of subclinical mastitis by indirect tests

S/N	Tests used	No. of cows Tested	No. of positive tests	Prevalence (%)	Overall Prevalence (%)	Chi-square (χ^2) value	P value
1	CMT*	280	119	42.50	41.96	0.066	0.79 (NS)
2	SFMT*	280	116	41.42			

**CMT= California Mastitis Test, *SFMT= Surf Field Mastitis Test, S/N= Serial Number, NS= Non-significant, χ^2 value= Chi square value, P value= Probability value*

4.1.1.1 Breed wise prevalence

The prevalence of SCM was significant (p<o.o5) between crossbreed and indigenous dairy cows where they showed 44.89% and 25.71% prevalence respectively (Table 3). Cross breed cows with well-formed udder and teat showed more prevalence. Udder and teat morphology is very heritable and could serve as a marker trait for selection to reduce mastitis in dairy cattle

Table 3. Breed wise prevalence of SCM in dairy cows

Breed	No. of cows tested	No. of positive tests	Prevalence (%)	Chi-square (χ^2) value	P value
Crossbreed	245	110	44.89	4.612	0.032*
Indigenous	35	9	25.71		

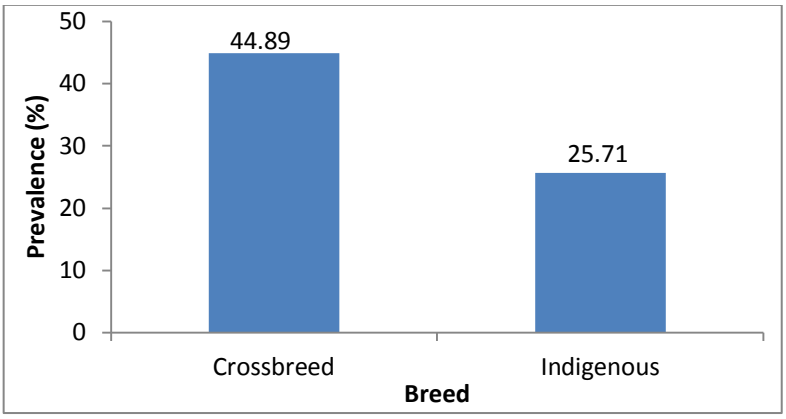

Figure 1. Breed wise prevalence of mastitis in dairy cows

4.1.1.2 Age wise prevalence

The distribution of SCM in different age group is presented in Table 4. The prevalence of SCM was higher ($p>0.05$) in the age group belonging to >12 years than others. The lowest prevalence (37.50%) was recorded in lower aged cows (2 to 3 years) and highest prevalence (47.05%) was recorded at higher aged cows (>12 years or above)

Table 4. Age wise prevalence of SCM in dairy cows

Age (Years)	No. of cows tested	No. of positive tests	Prevalence (%)	Chi-square (χ^2) value	P value
2 to 3	16	6	37.50	0.368	0.985 (NS)
>3 to 5	93	39	41.93		
>5 to 8	104	44	42.30		
>8 to 12	50	22	44.00		
>12 and above	17	8	47.05		

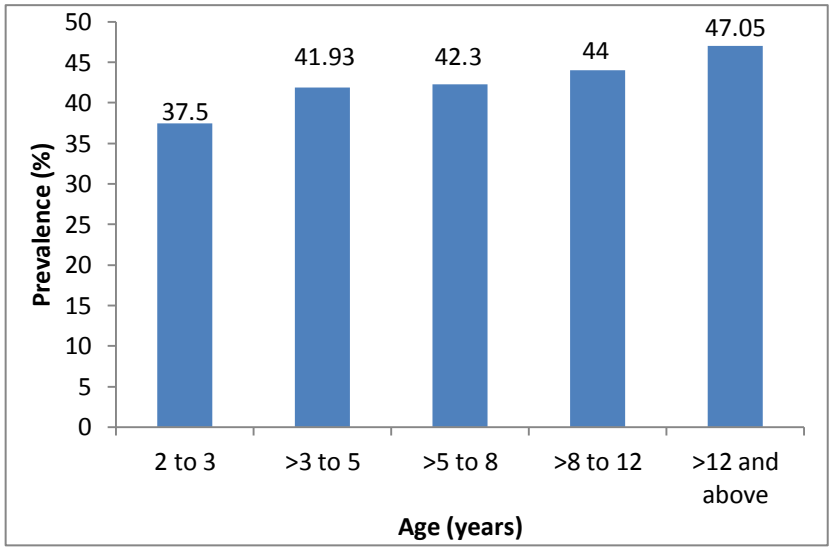

Figure 2. Age wise prevalence of mastitis in dairy cows

4.1.1.3 Lactation stage wise prevalence

It appears from the Table 5 that, there is a significant (P<0.05) association among cows of all three stages of lactation affected with SCM. The prevalence of SCM was recorded 50.41%, 38.73% and 31.25% during the early, mid and late stages of lactation, respectively. The prevalence gradually decreases with advancing of lactation period.

Table 5. Prevalence of SCM on the basis of lactation stage

Lactation stage	No. of cows tested	No. of positive tests	Prevalence(%)	Chi-square (χ^2) value	P value
Early (1-3 month)	121	61	50.41	6.229	0.044*
Mid (>3-6 months)	111	43	38.73		
Late (>6 months)	48	15	31.25		

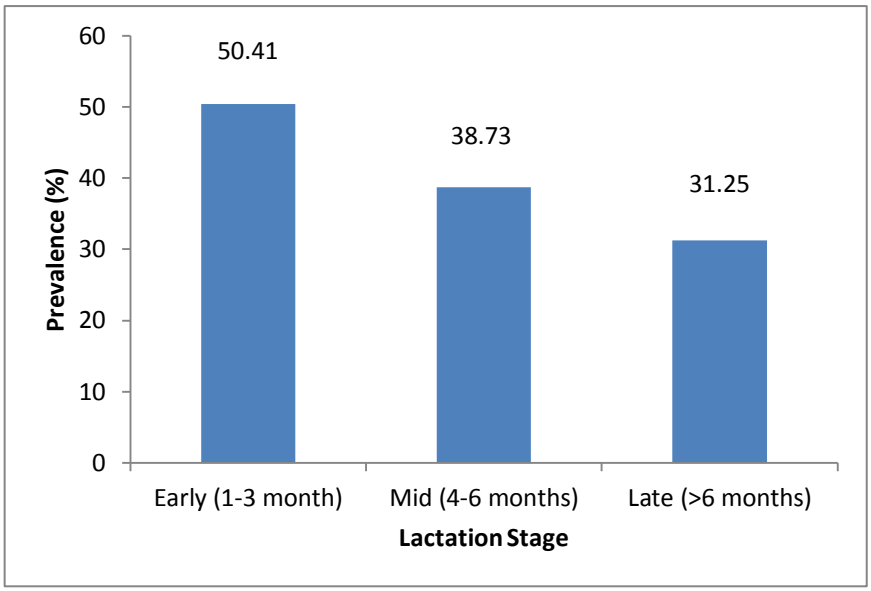

Figure 3. Lactation stage wise prevalence of mastitis in dairy cows

4.1.1.4 Parity Related Prevalence

The prevalence of SCM 30.35%, 36.11%, 41.26%, 52.94% and 60.52% during the parity number 1,2,3,4,5 and above, respectively (Table 6). There is significant ($P<0.05$) association where prevalence is higher with the increasing number of parity.

Table 6. Parity related prevalence of SCM in dairy cows

Parity	No. of cows tested	No. of positive tests	Prevalence (%)	Chi-square (χ^2) value	P value
1st	56	17	30.35	11.94	0.018*
2nd	72	26	36.11		
3rd	63	26	41.26		
4th	51	27	52.94		
5th and above	38	23	60.52		

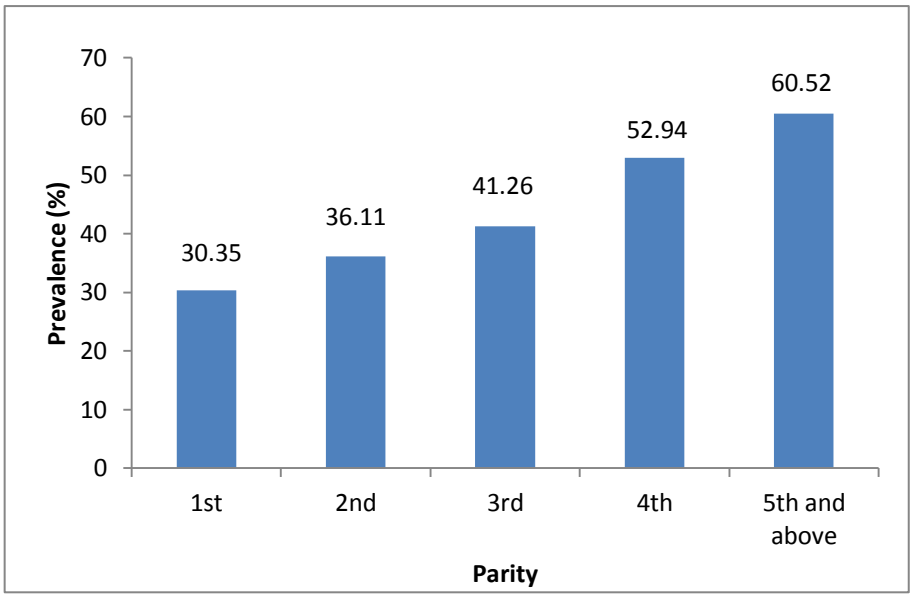

Figure 4. Parity related prevalence of mastitis in dairy cows

4.1.1.5 Milk Yield Related Prevalence

The prevalence of SCM 25.64%, 44.39% and 66.66% in low, medium and high milk producing cows, respectively (Table 7). There is significant ($P<0.05$) association where prevalence is higher with the increasing milk production.

Table 7. Prevalence of SCM related to milk yield

Milk yield (Liters)	No. of cows tested	No. of positive tests	Prevalence (%)	Chi-square (χ^2) value	P value
Low (1-5 lit)	39	10	25.64	7.028	0.030*
Medium (>5 to 10 lit)	232	103	44.39		
High (>10 lit)	9	6	66.66		

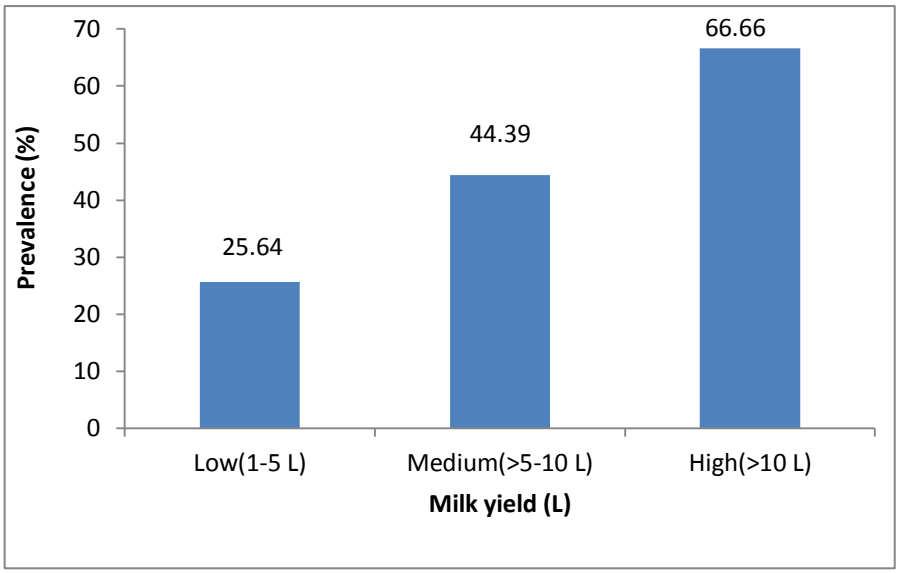

Figure 5. Milk yield related prevalence of mastitis in dairy cows

4.2 Necropsy Findings of Clinical Mastitis

Among all cows in the study, investigated for any gross injury on udder or teat, most of them revealed udder or teat gross lesion as shown in Chapter III. Some of the main gross udder/teat lesions and teat end lesions observed during examination by naked eye were witnessed by pictures as labelled Photo 5,6,7,8 and 9 (Chapter III). Abnormal color and consistency of milk and presence of blood in milk were observed (Photo 4 and 5). Abnormal conformation of different udder quarters, abnormal size and shape of teats, discoloration of udder, pus formation, hyperkeratosis and laceration at different parts of udder were recorded. Among the gross udder and teat lesions encountered in the present study, skin nodule and teat blindness were most prevalent (Photo 7 and 8).

The cut sections of mammary gland of mastitic cow shows different gross pathological lesions as it is shown on Photo 13 and subsequent photos there of reveal gross lesion and cut section of clinical mastitic cow. The cut section of affected teat canal showed abnormal tissue growth (Photo 14) and accumulation of pus and cellular debrises in the streak canal (Photo 14 and 17). Those abnormal tissues of teat canal were fragile and released watery materials by digital pressure. Varying degrees of haemorrhage at the mucosa of teat canal was also noted (Photo 15). Nodule formation at different parts of glandular tissue and teat canal were also visible (Photo 16 and 18).

Photo 13. Cut section of a mastitic udder

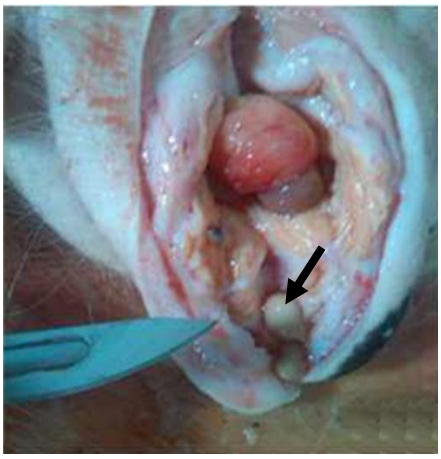

Photo 14. Pus accumulation in the streak canal along with abnormal tissue growth

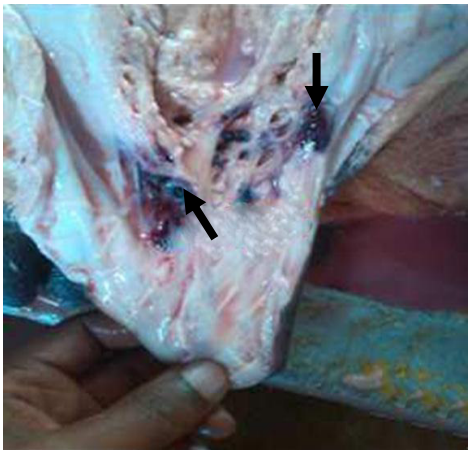

Photo 15. Haemorrhagic mucosa of teat canal

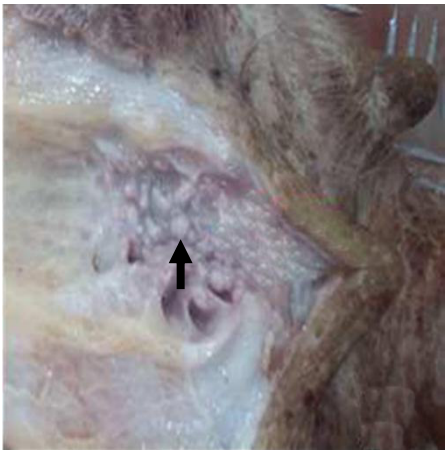

Photo 16. Nodule formation at the base of teat canal

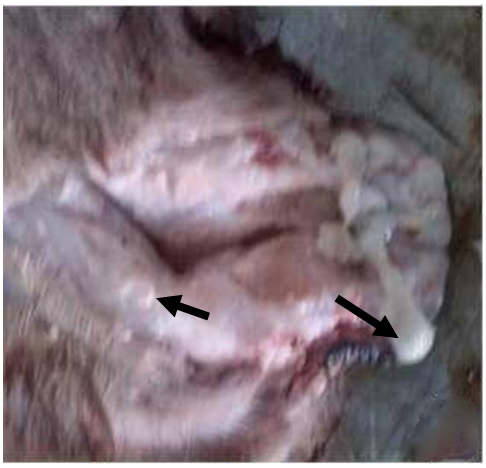

Photo 17. Pus accumulation in the gland

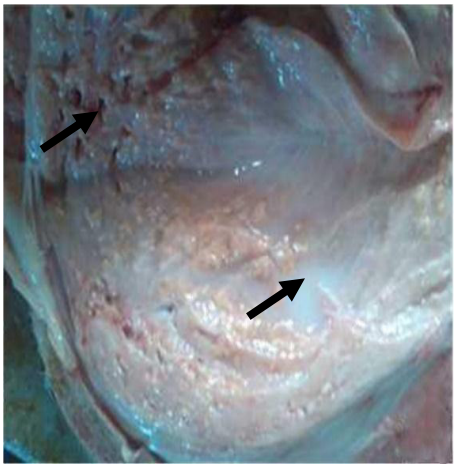

Photo 18. Cut section of glandular tissue with extensive nodular formation

4.3 Histopathological Findings of Clinical Mastitis

Histopathologically, the udder section from healthy cattle showed no pathological lesions. However, the tissue sections from mastitic animals revealed mild, moderate or severe atrophy of alveoli (Photo 21). The alveoli of affected udder tissue contained pink staining coagulated protein precipitates (Photo 19). The cellular exudate in udder tissue was present in the lumen of the alveoli in varying amounts in a number of cases. Infected mammary parenchymal tissues showed destruction of alveoli and fibrous tissue proliferation (Photo 20). Cellular infiltration mainly was observed in different areas of mammary tissues (Photo 22). Mastitic udder showed significantly lower alveolar epithelial cell population, alveolar luminal diameter and number of alveoli per plate.

Major histopathological findings during microscopic observation of collected sample from mastitic udder revealed following findings:

- Mass destruction of udder parenchyma, milk alveoli and alveolar atrophy
- Precipitates of coagulated protein was found predominantly in milk alveoli
- Huge infiltration of reactive cells
- Fibrous tissue proliferation
- Homogenous pink staining proteinaceous fluid was observed

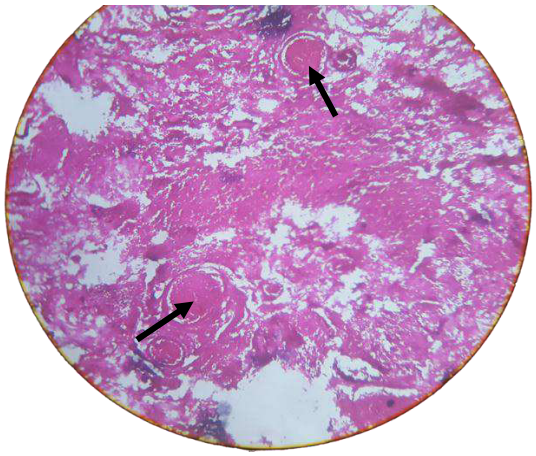

Photo 19. Coagulated protein precipitates in the milk alveoli

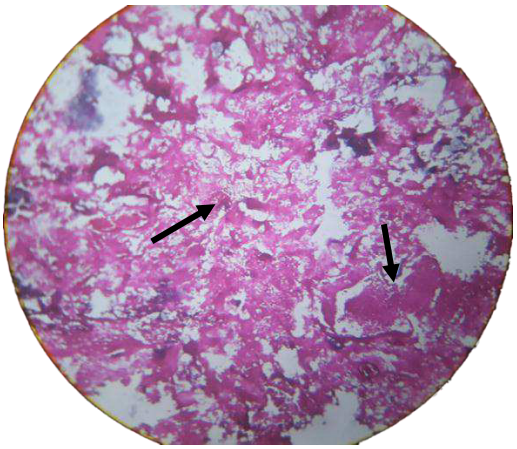

Photo 20. Mass destruction of alveoli along with fibrous tissue proliferation

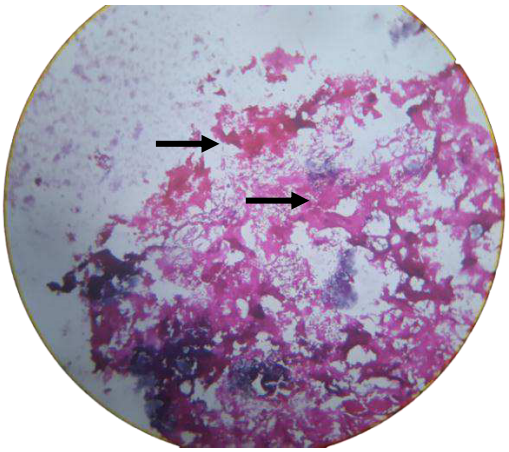

Photo 21. Mass destruction of udder parenchyma with alveolar atrophy

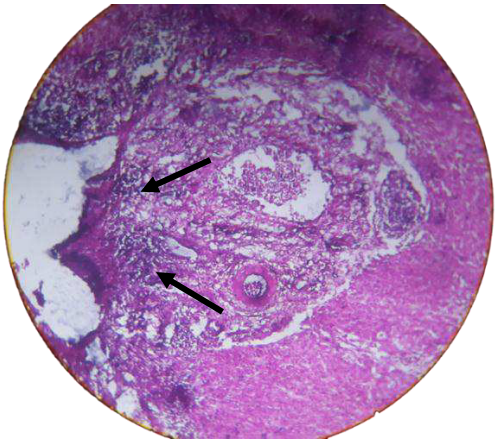

Photo 22. Huge infiltration of reactive cells

CHAPTER V
DISCUSSION

Mastitis is a complex disease involving interactions of several factors, mainly of management, environment, and factors relating to animal age, breed, stage of lactation, parity, milk yield and causative organisms. Its prevalence is expected to vary from place to place. And also variations in husbandry practices between different areas might, at least, partly explain the difference in prevalence reported by different authors.

5.1 Overall Prevalence of Subclinical Mastitis

The prevalence of subclinical mastitis was recorded 42.50% (CMT) and 41.42% (SFMT) where CMT showed higher prevalence than SFMT. Overall prevalence of subclincal mastitis by indirect tests was 41.96%. Barua *et al.* (2014) found the prevalence of SCM by CMT and SFMT were 32.43% (n=144) and 31.53% (n=140) respectively which is lower but somewhat similar to this study. In contrast, the current finding is greater than Nibret *et al*. (2011) who reported 32.6% and 33.6% prevalence of SCM by indirect tests. Islam *et al.* (2010) reported that 37.58% (n=124) and 35.15% (n=116) cows showed positive reaction for SCM by CMT and SFMT respectively. Islam *et al.* (2011) found the overall prevalence of SCM was 36.46% and CMT showed better performance in detecting SCM (37.58%) among the indirect tests used. Overall prevalence of subclinical mastitis (SCM) in lactating dairy cows found in this study was 29%. Cows were infected with SCM 29.5% and 25.5% detection by CMT and SFMT respectively. However, the high prevalence of sub-clinical mastitis may be attributed to improper milking hygiene, lack of post milking teat dipping and contact labours used, absence of order in milking cows of different ages and milking of mastitic animals before the healthy ones all of which might have increased the prevalence.

5.1.1 Breed wise prevalence

The prevalence of SCM was significant ($p<0.05$) between crossbreed and indigenous dairy cows where they showed 44.89% and 25.71% prevalence respectively. In this study, prevalence is more in cross breed than indigenous dairy cattle. However, similar findings as this study also recorded by Rahman *et al.* (2009). Islam *et al.* (2011) reported that all the cows of BAUDF were cross breed and show 37.5% positive reaction whereas indigenous and cross breed of Tangail sadar Upazila show 24.6% and 36.66% positive

reaction. Of 70 crossbred cows screened for SCM showed 37.5% and 36.66% in Bangladesh Agricultural University Dairy Farm (BAUDF) and in Tagail sadar upazial whereas local breed cows showed 24.61% positive reaction for SCM.

This may be attributed to vigorous udder and teat conformation and more milk production by cross breed cows than that of indigenous cows. The udder teats are the first line of defence against intra-mammary infection. The probability of mastitis occurring varies considerably between different teat shapes, sizes, teat placement and the morphology of the teat tips. The cross breed cows are more prone to causative organisms because of favorable conformation of teat.

5.1.2 Age wise prevalence

The distribution of SCM in different age group is presented in Table 4. The prevalence of SCM was higher ($p>0.05$) in the age group belonging to more than 12 years than others. In this study, the lowest prevalence (37.50%) was recorded in lower aged cows (2 to 3 years) and highest prevalence (47.05%) was recorded at higher aged cows (>12 years or above).

Islam *et al.* (2010) reported that The prevalence of SCM was significantly ($p<0.01$) higher (47.61%) in the age group belonging to more than 13 years than others. The prevalence of clinical mastitis was also increased in older cows. Similar observation was also reported by Rahman *et al.* (2009). Islam et al. (2011) also reported that The prevalence of SCM was recorded as 22.22%, 27.94%, 21.21% and 18.18%, respectively at the age group of 3 years to 5 years, >5 years to 8 years, >8 years to 12 years and >12 years in local breed cows. The prevalence of SCM was recorded 33.33%, 40.90%, 28.57% and 0.00% respectively at the age group of 3 years to 5 years, >5 years to 8 years, >8 years to 12 years and >12 years in cross breed cows.

This may be due to older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder. The prevalence of mastitis is higher in adult aged cows than the young cows and the present report of this study also supported by the report of Rasool *et al.* (1985) who reported that rate of occurrence of mastitis increased with age.

5.1.3 Lactation stage wise prevalence

In this study, the prevalence of SCM was recorded 50.41%, 38.73% and 31.25% during the early, mid and late stages of lactation, respectively. The prevalence gradually decreases with advancing of lactation period. The result of this study agrees with Pal and Verma (1988) who reported lower prevalence of SCM in the stage of lactation above five months. However, this result varies with Islam *et al.* (2010) who reported that the prevalence of SCM was highest in late lactation (72.45%) followed by early (40%) and mid lactation (27.56%) which is comparable to the findings of Rabbani and Samad (2010). The prevalence of mastitis in cows were early, mid, late stage was 30%, 17.14% and 25.45% respectively by the report of Kivaria *et al*. (2014).

This may be due to more milk production at early stage than that of mid and late stage. However, the teat opening remains flexible and favorable at early stage of lactation which tends to more prone to microbial attack.

5.1.4 Parity Related Prevalence

The prevalence of SCM of this study was 30.35%, 36.11%, 41.26%, 52.94% and 60.52% during the parity number 1,2,3,4,5 and above, respectively. In this study, cows with increasing parity number had significantly higher prevalence than lesser number of parity ($p<0.05$). Islam *et al.* (2010) reported that there is significant ($P<0.05$) association where prevalence is higher with the increasing number of parity. Significantly ($p<0.01$) higher prevalence (50%) of SCM was recorded at the parity group of more than11. Similarly prevalence of clinical mastitis was also increased with increased number of parity of cows. This observation supports the reports of Rasool *et al.* (1985); Devi *et al.* (1997) and Dego and Tareke (2003). Barua *et al.* (2014) found that parity 4 to rest showed greater prevalence (48.98%) of mastitis than parity 1 to 3 (37.50%). Prevalence (53.50 %) was higher in pregnant cows than fresh cows (29.38%). Advancing age (9-18 years) showed higher prevalence (45.65%) than lower age (3-8 years) which was (38.07%).

This could be due to that fact that primiparous cows have more effective udder defence mechanism than multiparous cows.

5.1.5 Milk Yield Related Prevalence

The prevalence of SCM 25.64%, 44.39% and 66.66% in low, medium and high milk producing cows, respectively (Table 7). There is significant ($P<0.05$) association where prevalence is higher with the increasing milk production. Barua *et al.* (2014) reported high milk producing cows showed greater prevalence (52.94%) than medium (35.00%) and low producing cows (37.90%). Similar findings were also reported by Islam et al. (2010) where prevalence of SCM was significantly ($p<0.05$) higher (37.12%) in high (>10 L) yielding cows than low to medium yielders. This finding was in agreement with the reports of Kader *et al.* (2003).

This may be attributed to cows with pendulous udders producing more milk had the highest risk of mastitis. High yielding cows often possess vigorous, round and pendulous udder. Many previous studies in different parts of the world have shown higher prevalence of mastitis in cattle having pendulous, round and bowl and long udder.

5.2 Gross Pathological Investigation

Position, size, shape and anatomical feature of udder, the dairy cows are susceptible to infection and injuries, which results in mastitis. The morphology of teat, especially apex and streak canal are recognized as parts of the passive defence mechanisms against intramammary infection (Shukla *et al.,* 1997). Anatomical characteristics of dairy cattle are not equal for all breeds, in a way that the udder and teat morphology could favour an individual performance or a determined breed. Due to these differences, some anatomical measurements of mammary glands have been used in research with dairy herds. In the case of improved breeding, the cow's udder has to undergo rapid changes in relation to size, position and adjustment for rapid removal of large volume of milk and such it is prone to injury and infection (Zwald *et al.,* 2004).

Higher prevalence of mastitis in impaired teat and long udder shape has been reported Klaas *et al.* (2004) and Bhutto *et al.* (2010). The logistic procedure also confirmed that teat lesions, teat/udder shape and pendulous udder were significantly associated with mastitis. The association of pendulous udder with teat/udder injuries has also been reported previously by Shukla *et al.* (1997) and Bhutto *et al.* (2010). Shukla *et al.* (2005) examined udder for injury, pain, swelling, hardness and fibrosis. The shape was

categorized into funnel, round, flat and plate types, while on the basis of length, teats were classified as small (<5.5 cm), medium (<5.5-7.5 cm) and large (>7.5 cm).

Zwald (2004) also found in his research that among the gross lesions, skin nodules and teat blindness were the top ranking having percentage of 22.68% and 11.5% respectively in this research. However, high occurrence of mastitis-induced blind mammary quarters, like the report of Biffa *et al.* (2005), which has a direct influence on milk production, signifies the importance of the problem. Lack of screening and treatment of subclinical mastitis and inadequate follow-up of clinical and chronic cases coupled with persistent challenges of the mammary glands by microbial pathogens could be the main predisposing factors to quarter blindness. This hidden and gradual destruction of the mammary tissues would end with non-functional quarters.

5.3 Histopathological Investigation

Microscopical examination of tissue sections from teat of infected cows exhibited severe histological changes. Histological analyses have been widely used since the 1970s and are still being used today for assessing damage to secretory tissue in the bovine mammary gland caused by mastitis pathogens (Stabemfeldt and Spencer, 1965; Zarkower and Norcross, 1966; Benites *et al.,* 2002). The most common histopathological findings in the present study were severe cellular infiltration, atrophy of alveoli, broken alveoli, increased stromal tissue (fibrosis) and presence of lymphoid nodules in alveoli.

The histopathological changes including number of alveoli, alveolar diameter and secretory alveolar cell population significantly decreased in mastitic cattle. These results indicated pathological changes occurring in udder tissue and could be due to severe tissue damage due to different mastitis pathogens. In accessible literature, no reports were found about number of alveoli and alveolar cell population in naturally mastitic animals.

The weak to negligible activity of protein in tissue sections of mastitic cattle in present study could be due to degenerative changes present in the parenchymatous cells, atrophy of lactiferous acini along with connective tissue proliferation and impaired activity of endoplasmic reticulum. No reports are available about the density of protein staining in mastitic cattle. However, different workers reported that protein staining was decreased in advanced stages of lactation (Hassan, 2004; Elsayed *et al.,* 2009). The weak enzyme

activity, low protein expression and lower alveolar secretary cell population in udder during involutionary stage have been reported (Elsayed *et al.,* 2009).

The present microscopic lesions of the mammary gland were in line with many of the previous works. The infected mammary gland showed inflammatory lesions of different types ranging from the disappearance of the alveolar lumen, through fibrosis to the complete destruction of the parenchyma (Kheira and Abdellatif, 2014).

Benites *et al.* (2002) examined 131 mammary parenchyma from 184 slaughtered dairy cows for existence of microorganisms and histopathological changes. Of all the samples from which microorganisms were isolated, 96.9% of samples showed inflammatory response.

According to Hussain *et al.* (2012) the tissue sections from mastitic animals revealed mild, moderate or severe atrophy of alveoli with cellular exudate in the lumen of the alveoli. The existence of acute and chronic inflammation in mammary parenchymal tissues was confirmed and fibrous tissue proliferation was seen in the mammary gland. Moreover, lesions of the breast tissue reduces the number and activity of epithelial cells and therefore contributes to lower milk production with increasing proportions of lymphocytes and macrophages was reported by Zhao and Lacasse (2007).

CHAPTER VI
CONCLUSIONS

Mastitis is a common problem of dairy cows and has a major economic importance in dairy industries. In this study, significantly a higher prevalence of subclinical mastitis in dairy cows as determined by CMT and SFMT was an indicative of a health and production problems in the smallholder dairy cows farmer in the study area. This study reveals that the different breeds of cows, age groups, stages of lactation, number of parity and level of milk yield are some important factors associated with the prevalence of SCM in dairy cows. Clinical inspection, necropsy and histopathological findings of this study may be implicated in the selection of dairy animals and better understanding of the pathological consequences of mastitis. However, mastitis is complex multi-causative agent disease that needs strategic control program through early detection. Continuous screening of cows at farm level and appropriate treatment should be implemented to reduce the loss. For controlling mastitis, hygiene should be maintained at every aspect and husbandry practices should be improved at household and farm level.

REFERENCES

Akers, R. M., & Nickerson, S. C. (2011). Mastitis and its impact on structure and function in the ruminant mammary gland. *Journal of mammary gland biology and neoplasia*, *16*(4), 275-289.

Awaz, K. B. (1997). Rapid Spot (MAUM) Test Based on Detection of Trypsin Inhibitors in Milk to Diagnose Bovine Subclinical Mastitis. *International Journal of Animal Sciences*, *12*, 97-101.

Barua, M., Prodhan, M. A. M., Islam, K., Chowdhury, S., Hasanuzzaman, M., Imtiaz, M. A., & Das, G. B. (2014). Sub-clinical mastitis prevalent in dairy cows in Chittagong district of Bangladesh: detection by different screening tests. *Veterinary World*, *7*(7) 483-488.

Bengtsson, B., Unnerstad, H., Ekman, T., Persson Waller, K., Lindberg, A., Artursson, K., & Nilsson Öst, M. (2005). Prevalence and antimicrobial susceptibility of bacteria causing acute clinical mastitis in dairy cows in Sweden 2002–03. *Mastitis in dairy production. Current knowledge and future solutions*, p. 888.

Benites, N. R., Guerra, J. L., Melville, P. A., & Da Costa, E. O. (2002). Aetiology and histopathology of bovine mastitis of espontaneous occurrence. *Journal of Veterinary Medicine, Series B*, *49*(8), 366-370.

Bhutto, A. L., Murray, R. D., & Woldehiwet, Z. (2010). Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. *The Veterinary Journal*, *183*(1), 63-67.

Biffa, D., Debela, E., & Beyene, F. (2005). Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. *International Journal of Applied Research in Veterinary Medicine*, *3*(3), 189-198.

Bilal, M. Q., & Ahmad, A. (2004). Dairy hygiene and disease prevention Usman and Bilal printing linkers, Faisalabad, Pakistan.

Blowey, R. & Edmondson, P. (1995). Mastitis control in dairy herds, an illustrated and practical guide. Farming press books, Ipswich, UK.

Colditz, I. G., & Watson, D. L. (1982). Effect of immunisation on the early influx of neutrophils during staphylococcal mastitis in ewes. *Research in Veterinary Science*, *33*(2), 146-151.

Dego, O. K., & Tareke, F. (2003). Bovine mastitis in selected areas of southern Ethiopia. *Tropical animal health and production*, *35*(3), 197-205.

Devi BK, Shukla PC and Bagherwal RK (1997). Incidence of sub-clinical mastitis in cows. *Indian Journal of Dairy Science* 50, 477-478.

Elsayed, E. H., El-Shafie, M. H., Saifelnasr, E. O. H., & El-Ella, A. A. (2009). Histological and histochemical study on mammary gland of Damascus goats through stages of lactation. *Small Ruminant Research*, *85*(1), 11-17.

Emanuelson, U., Olsson, T., Holmberg, O., Hageltorn, M., Mattila, T., Nelson, L., & Åström, G. (1987). Comparison of some screening tests for detecting mastitis. *Journal of Dairy Science*, *70*(4), 880-887.

Erskine R. J. (2001). Mastitis control in dairy herds. In: Radstits, O. M. (Ed.): Herd following diagnosis of mastitis detected by a change in the electrical conductivity of milk. J. Dairy Sci., 80, 859-863.

Fox, L. K., & Gay, J. M. (1993). Contagious mastitis. *The Veterinary clinics of North America. Food animal practice*, *9*(3), 475-487.

Guidry, A .J. (2007). Mastitis and the immune system of the mammary gland. In: Larson, B.L. (eds): *Lactation*. The Iowa State University Press, Ames, Iowa, USA, Pp. 229-262.

Gulyas, L. and Ivancsics, J. (2002). Relationship between the somatic cell count and certain udder-morphologic traits. *Acta Veterinaria Hungarica*, 50: 373–383.

Hassan, L. R. (2004). Histochemical assessment of mammary gland capacity pertaining to milk production in buffaloes. *Egyptian Journal of Animal Production*, 41, 309-320.

Holdway, R. J. (1992). Bovine Mastitis in New Zealand Dairy Herds: Part Ill. The Cost of Mastitis to the New Zealand Dairy Farmer during the 199 111992 Dairy Season. *Unpublished report to the Livestock Improvement Corporation, Hamilton*.

Houe, H., Vaarst, M., & Enevoldsen, C. (2002). Clinical parameters for assessment of udder health in Danish dairy herds. *Acta veterinaria Scandinavica*, *43*(3), 173-184.

Hussain, M., Ghafoor, A., & Saboor, A. (2010). Factors affecting milk production in buffaloes: a case study. *Pakistan Veterinary Journal*, *30*(2), 115-117.

Hussain, R., Javed, M. T., Khan, A., Mahmood, F., & Kausar, R. (2012). Mastitis and associated histo-pathological consequences in the context of udder morphology. *International Journal of Agriculture and Biology*, *14*(6), 947-952.

Islam, M. A., Islam, M. Z., Islam, M. A., Rahman, M. S., & Islam, M. T. (2011). Prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *9*(1), 73-78.

Islam, M. A., Rahman, A. K. M. A., Rony, S. A., & Islam, M. S. (2010). Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. *Bangladesh Journal of Veterinary Medicine*, *8*(2), 157-162.

Javaid, S. B., Gadahi, J. A., Khaskeli, M., Bhutto, M. B., Kumbher, S., & Panhwar, A. H. (2009). Physical and chemical quality of market milk sold at Tandojam, Pakistan. *Pakistan Veterinary Journal*, *29*(1), 125-127.

Kader, M. A., Samad, M. A., & Saha, S. (2003). Influence of host level factors on prevalence and economics of subclinical mastitis in dairy milch cows in Bangladesh. *Indian Journal of Dairy Science*, *56*(4), 235-240.

Kheira, G., & Abdellatif, N. (2014). Impact of subclinical mastitis on the health of the mammary gland. *Global Veterinaria*, *12*(2), 193-196.

Kivaria, F. M., Noordhuizen, J. P. T. M., & Kapaga, A. M. (2004). Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Tropical Animal Health and Production*, *36*(6), 581-592.

Klaas, I. C., Enevoldsen, C., Vaarst, M., & Houe, H. (2004). Systematic clinical examinations for identification of latent udder health types in Danish dairy herds. *Journal of Dairy Science*, *87*(5), 1217-1228.

Luna, L. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York. Available at: http://agris.fao.org/agris-search/search.do?recordID=US201300459165.

Lundberg, Å. (2015). Mastitis in Dairy Cows: Genotypes, Spread, and Infection Outcome of Three Important Udder Pathogens. Swedish University of Agricultural Sciences, 28, 1-89.

Majic, B., Jovanovic, B. V., Ljubic, Z., & Kukovics, S. (1993). Typical problems encountered in Croatia in the operation of goats milking machines. In *Proceedings of the 5th Internacional symposium on machine milking of small ruminants. Budapest, Hungary,* pp. 377-379.

Middleton, J. R., Hardin, D., Steevens, B., Randle, R., & Tyler, J. W. (2004). Use of somatic cell counts and California mastitis test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. *Journal of the American Veterinary Medical Association*, *224*(3), 419-423.

Milner, P., Page, K. L., & Hillerton, J. E. (1997). The effects of early antibiotic treatment following diagnosis of mastitis detected by a change in the electrical conductivity of milk. *Journal of dairy science*, *80*(5), 859-863.

Muhammad, G., Athar, M., Shakoor, A., Khan, M. Z., Rehman, F., & Ahmad, M. T. (1995). Surf Field Mastitis Test: An inexpensive new tool for evaluation of wholesomeness of fresh milk. *Pak. J. Food Sci*, *5*(3-4), 91-93.

Nooruddin M, Ali ML and Debnath NC (1997). Retrospective epidemiologic study of periparturient diseases in dairy cows. 1. Clinical mastitis. *The Bangladesh Veterinarian* 14, 43-47.

Ogorevc, J., Kunej, T., Razpet, A., & Dovc, P. (2009). Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal genetics*, *40*(6), 832-851.

Oviedo-Boyso, J., Valdez-Alarcón, J. J., Cajero-Juárez, M., Ochoa-Zarzosa, A., López-Meza, J. E., Bravo-Patino, A., & Baizabal-Aguirre, V. M. (2007). Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. *Journal of Infection*, *54*(4), 399-409.

Pal, P., Verma, B. B., & Prasad, R. S. (1988). Some observation on efficacy of indirect test for detection of sub-clinical mastitis. *Indian Journal Veterinary Medicine*, *8*, 40-41.

Pösö, J., & Mäntysaari, E. A. (1996). Relationships between clinical mastitis, somatic cell score, and production for the first three lactations of Finnish Ayrshire. *Journal of Dairy Science*, *79*(7), 1284-1291.

Putt, S. N. H. (1987). *Veterinary epidemiology and economics in Africa: a manual for use in the design and appraisal of livestock health policy* (Vol. 3). ILRI (aka ILCA and ILRAD), p. 130.

Rabbani, A. F. M. G., & Samad, M. A. (2010). Host determinants based comparative prevalence of subclinical mastitis in lactating Holstein-Friesian cross cows and Red Chittagong cows in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *8*(1), 17-21.

Radostits, O. M., Leslie, K. E., & Fetrow, J. (1994). *Herd Health: Food Animal Production Medicine,* 2, 631-638.

Rahman, M. A., Bhuiyan, M. M. U., Kamal, M. M., & Shamsuddin, M. (2009). Prevalence and risk factors of mastitis in dairy cows. *Bangladesh Veterinarian*, *26*(2), 54-60.

Rainard, P. and Riollet, C. (2006). Innate immunity of the bovine mammary gland. *Veterinary Research, 37*, 369-400.

Rasool, G., Jabbar, M. A., Kazmi, S. E., & Ahmad, A. (1985). Incidence of sub-clinical mastitis in Nili-Ravi buffaloes and Sahiwal cows. *Pakistan Veterinary Journal, 5,* 76–78.

Rodenburg, J. (1990). Mastitis Prevention: Environmental Control: Ontario. *Ministry of Agriculture and Food Factsheet.* AGDEX 410/662.

Samad, M. A. (2008). Animal Husbandry and Veterinary Science, volume II, LEP pub no. 11, Bangladesh Agricultural University campus. pp. 184-1189.

Seffner, W., & Pfützner, H. (1980). Mycoplasma mastitis of cattle. 8. Pathological anatomy and histology of experimental Mycoplasma bovis mastitis. *Archiv fur experimentelle Veterinarmedizin*, *34*(6), 817-826.

Sharma, S., & Singh, K. B. (2006). Investigations of teat skin lesions in dairy cattle in Punjab. *Indian Veterinary Journal*, *83*(10), 1081-1083.

Shukla, S. K., Dixit, V. P., & Chandra, R. (2005). An epidemiological study of mastitis in an organized dairy farm. *Indian Journal of Veterinary Medicine*, *25*(2), 118.

Shukla, S. K., Dixit, V. P., Thapliyal, D. C., Garg, S. K., & Kumar, A. (1997). A note on the incidence of bovine mastitis in relation to teat shape, size and quarters affected. *Indian Veterinary Journal*, *74*(11), 989-990.

Singh, P. J., Singh, K. B., Jand, S. K., Dhingra, P. N., & Nauriyal, D. C. (1994). Incidence, etiology and antibiogram of pathogens isolated from subclinical mastitis in machine milked cows. *Indian Journal of Dairy Science*, *47*(9), 730-733.

Smith, K. L., & Hogan, J. S. (1993). Environmental mastitis. *Veterinary Clinics of North America: Food Animal Practice*, *9*(3), 489-498.

Sol, J., Sampimon, O. C., Snoep, J. J., & Schukken, Y. H. (1994). Factors associated with bacteriological cure after dry cow treatment of subclinical staphylococcal mastitis with antibiotics. *Journal of Dairy Science*, *77*(1), 75-79.

Sordillo, L. M., & Streicher, K. L. (2002). Mammary gland immunity and mastitis susceptibility. *Journal of Mammary Gland Biology and Neoplasia*, *7*(2), 135-146.

Stabenfeldt, G. H., & Spencer, G. R. (1965). The lesions in bovine udders shedding hemolytic coagulase-positive staphylococci. *Pathologia Veterinaria*, *2*(6), 585-600.

Szencziová, I., Strapák, P., Stádník, L., Ducháček, J., & Beran, J. (2013). Relationship of udder and teat morphology to milking characteristics and udder health

determined by ultrasonographic examinations in dairy cows. *Annals of Animal Science*, *13*(4), 783-795.

Tančin, V., Uhrinčať, M., Mihina, Š., Sudzinová, J., Foltys, V., & Tančinová, D. (2007). Somatic cell count and quarter milk flow parameters from udder of dairy cows. *Slovak Journal of Animal Science*, *40*, 79-82.

Tilki, M., İNAL, Ş., Colak, M., & Garip, M. (2005). Relationships between milk yield and udder measurements in Brown Swiss cows. *Turkish Journal of Veterinary and Animal Sciences*, *29*(1), 75-81.

Trinidad, P., Nickerson, S. C., & Adkinson, R. W. (1990). Histopathology of Staphylococcal Mastitis in Unbred Dairy Heifers1. *Journal of dairy science*, *73*(3), 639-647.

Zarkower, A., & Norcross, N. L. (1966). Histological changes in the bovine mammary gland after infusion with Streptococcus agalactiae extract. *The Cornell Veterinarian*, *56*(4), 555-566.

Zdunczyk, S., Zerbe, H., & Hoedemaker, M. (2003). Importance of estrogens and estrogen-active compounds for udder health in cattle. A review. *DTW. Deutsche tierarztliche Wochenschrift*, *110*(11), 461-465.

Zeleke, M. M., & Bishoftu, E. (2016). Morphometric, pathological and bacteriological study of mammadry gland of cows with subclinical and clinical mastisis in selected farms and abattoir in central Ethiopia Addis Ababa (Doctoral Dissertation, Addis Ababa University).

Zhao, X., & Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: Causes and control 1. *Journal of Animal Science*, *86*(13_suppl), 57-65.

Zwald, A. G., Ruegg, P. L., Kaneene, J. B., Warnick, L. D., Wells, S. J., Fossler, C., & Halbert, L. W. (2004). Management Practices and Reported Antimicrobial Usage on Conventional and Organic Dairy Farms1. *Journal of Dairy Science*, *87*(1), 191-201.