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Thomby Paul
June, 2022

CLINICAL AND ULTRASONOGRAPHICAL EVALUATION OF MAMMARY GLAND DURING MASTITIS IN RUMINANTS

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Dedicated to

my beloved

parents

Acknowledgements

Table of Contents

List of figures

List of graphs

List of tables

Table No.	Title	Page No.
Table 4.1	The percentage of mastitis based on DMSCC at different levels in goats and cattle	30
Table 4.2	The ultrasonographical measurement of teat parameters in goats	39
Table 4.3	The ultrasonographical measurement of teat parameters in cattle	42
Table 4.4	The ultrasonographical measurement of supramammary lymph nodes in goats	55
Table 4.5	The ultrasonographical measurement of supramammary lymph nodes in cattle	55
Table 4.6	The measurement of haematological parameters in normal, subclinical and clinical mastitis in goats and cattle	62

List of abbreviations

Abbreviations	Elaboration
CVASU	Chattogram Veterinary and Animal Sciences University
SAQTVH	Shahedul Alam Quadery Teaching Veterinary Hospital
CMT	California Mastitis Test
%	Percentage
/	Per
<	Less Than
>	Greater Than
±	Plus or minus
°C	Degree Celsius
µl	Microlitre
B mode	Brightness Mode
CBC	Complete Blood Count
CM	Clinical Mastitis
cm	Centimeter
DLC	Differential Leukocyte Count
Ds	Dorsal
DMSCC	Direct Microscopic Somatic Cell Count
ESR	Erythrocyte Sedimentation Rate
et al.	et alibi or 'And others'
etc.	et caetera
Hb	Haemoglobin
Lt	Left
MHz	Mega Hertz
min.	Minute

ml	Milliliter
mm	Millimeter
mm^2	Square Millimetre
N	Number
PCV	Packed Cell Volume
RBC	Red Blood Cell
Rt	Right
SCC	Somatic Cell Count
SCM	Subclinical Mastitis
SD	Standard Deviation
TCD	Teat Canal Diameter
TcD	Teat Cistern Diameter
TCL	Teat Canal Length
TEC	Total Erythrocyte Count
TLC	Total Leukocyte Count
TWT	Teat Wall Thickness
Vt	Ventral

Abstract

Ultrasonography is an effective, non-invasive, non-ionizing and rapid method of detecting pathological changes in the mammary gland in ruminants. The study was designed to evaluate the clinical and ultrasonographical changes in mammary gland during mastitis in ruminants. The mammary glands of 40 lactating crossbred Jamnapari goats and 10 lactating crossbred Holstein Friesian cattle were examined clinically and ultrasonographically using a multi-frequency (5-10 MHz) linear transducer. Somatic cell count was performed on each collected milk sample from each quarter, and classified into three groups as normal, subclinical and clinical mastitis. In goats, 17 animals (42%) had normal, 13 animals (32%) had subclinical mastitis and 10 animals (25%) had clinical mastitis. In cattle, 2 animals (20%) had normal, 3 animals (30%) had subclinical mastitis and 5 animals (50%) had clinical mastitis. On clinical examination of udder in goats, both the subclinical and clinical mastitis were found more frequent in asymmetrical udders compared to symmetrical udders. Pendulous-shaped udders were more affected with both the subclinical and clinical mastitis than spherical-shaped udders. On the other hand, bottle-shaped and cylindrical-shaped teats were more prone to subclinical and clinical mastitis than funnel-shaped teats in goats. During the evaluation of udder and teats, visible abnormalities were observed in clinical mastitis such as swollen udder and teats, induration, pain on palpation, warm on touch and changes in color of the skin etc. Milk abnormalities including white clotted, bloody and watery milk were found in clinical mastitis in both species. The ultrasonographic measurement of teat structures revealed that teat canal length and teat canal diameter were significantly ($p<0.05$) shorter and narrower in normal animal than both the subclinical and clinical mastitis in goats. Ultrasonographically, clinical mastitis was characterized by a non-homogeneous and hypo to hyperechoic structures of the mammary parenchyma, and gland cisterns were found hypoechoic contents with lack of clear visualization of the lactiferous ducts. Irregularity of contour of the teat wall along with numerous hypo to hyperechoic structures were found in teat cisterns in both the goats and cattle affected with clinical mastitis. In addition, teat cisterns were found anechoic contents as well as somewhat irregularity of teat walls were revealed in normal animals as well as subclinical mastitis. The teat canal obstruction was found in clinical mastitis, where

the obstruction was more frequent in teats of cattle than in goats. The ultrasonographic length and width of the supramammary lymph nodes were significantly ($p< 0.05$) increased in both goats and cattle affected with clinical and subclinical mastitis compared to normal animals. During haematological examination of goats and cattle, hemoglobin, packed cell volume and total erythrocyte count were significantly different ($p<0.05$) among normal, subclinical and clinically affected animals. Total leukocyte count, the percentage of neutrophils and band cells were significantly ($p<0.05$) increased in clinical mastitis than the normal and subclinical mastitis in both goats and cattle. As the clinical and ultrasonographical evaluation are the true reflection of mastitis in ruminants, which may assist clinicians in predicting the prognosis of mastitis. Therefore, ultrasonography can be used as a fast complementary technique in field conditions for the proper diagnosis and treatment of mastitis in ruminants.

Keywords: Clinical evaluation, ultrasonography, mastitis, ruminants.

Chapter - 1: Introduction

Livestock is an integral component of agro-based economy in Bangladesh. The contribution of livestock in total Gross Domestic Product (GDP) is 1.90%, and GDP growth rate of livestock is 3.1% in 2021-2022. The population of livestock species in Bangladesh are: cattle 24.7 million, buffalo 1.5 million, sheep 3.8 million and goat 26.8 million according to Department of Livestock services in 2021-2022. Cattle and goats are the main source of meat production and dairy products in many areas in the world including Bangladesh (Haenlein, 2004 and Hossain et al., 2016). In Bangladesh, cattle and goat production have also raised significantly during the last decades due to great demand of meat, milk and skin. Dairy farming is considered as a tool in rural development programs for improving the socio-economic conditions of subsistence farmers and increase women empowerment (Kumar et al., 2018 and Datta et al., 2019). Although dairy farming plays a vital role in development of socio-economic conditions, it faces many challenges for its optimum production such as infectious and non-infectious diseases, improper husbandry practices, negligible artificial insemination practices and production of good quality animal, poor access to veterinary care and lack of marketing chain of milk (Hegde, 2019 and Akter et al., 2020). Milk production is very important for sustainable development of economy and ensure nutritive value of increasing populations where need to increase milk production and upgrade intensive rearing system (Datta et al., 2019), but it is hampered by different diseases like mastitis, reproductive problems, lameness, and different metabolic abnormalities (Britt et al., 1986; Vailes and Britt, 1990; Goldberg et al., 1992; Phillips et al., 2013).

Mastitis is one of the most baleful disease in dairy farming which significantly reduces not only quantity and quality of milk, but also losses farmer's economy (DeGraves and Fetrow, 1993; Koop et al., 2010; Razi et al., 2012 and Islam et al., 2019). Mastitis is the inflammation of mammary gland resulting from systemic hematogenous infection to udder or directly entry of pathogens through teat orifice to mammary parenchyma, where physical and/or chemical changes in milk composition and pathological alterations in glandular tissues (Radostits et al., 2007). Mastitis can be classified in different ways, depending on the presenting clinical signs in animal

that are subclinical and clinical mastitis. Different forms of clinical mastitis are seen in cattle and goats such as per-acute gangrenous form, acute form and chronic form (Radostits et al., 2007). Subclinical mastitis is characterized by alterations in chemical compositions of milk but there is no evidence of macroscopic changes in mammary gland (Constable et al., 2017). On the other hand, acute mastitis is characterized by presenting the signs of inflammation such as swelling, reddening, warming and touching to pain, and also visible changes in milk such flakes, clots etc. (Radostits et al., 2007; Smith and Sherman, 2009). There is also found enlargement of the supramammary lymph nodes, and animal shows reluctant to move (Machado, 2018). The chronic form of mastitis is characterized by secretory tissues of udder parenchyma are gradually converted into fibrous tissues, hard in consistency and shrunken than normal quarter. In case of per-acute gangrenous mastitis, the udder become swollen, bluish discoloration, fluid exudation and coldness in palpation. In severe conditions, subcutaneous emphysema and crepitating sounds are also found in affected mammary gland (Menzies and Ramanoon, 2001 and Abd-El-Hady, 2015).

The prevalence of clinical mastitis in goat was recorded 5.3% under rural condition in Bangladesh (Sarker and Samad, 2011) whereas the prevalence of clinical mastitis in cattle was recorded 55% in Chattogram (Jha et al., 2010). The prevalence of subclinical mastitis in goat was recorded 50.9% based on CMT test under Chattogram metropolitan city in Bangladesh (Akter et al., 2020) and the prevalence subclinical mastitis in dairy cows in Chattogram district of Bangladesh was reported 32.43% based on CMT (Barua et al., 2014). Mastitis is predisposed by several risk factors at farm levels (environmental hygiene, management etc.), animal levels (breed, age, BCS, parity, lactation stages etc.) and quarter levels (udder shape, udder depth, teat shape, teat length, teat end shape etc.) (James et al., 2009; Koop et al., 2016 and Akter et al., 2020). A variety of infectious agents such as bacteria, virus and fungi are responsible for mastitis in ruminants (Watts, 1988; Menzies and Ramanoon, 2001; Bradley et al., 2001; Tiwari et al., 2013 and Horpiencharoen et al., 2019). The pathogens causing mastitis are commonly classified into two groups: i) contagious pathogens and ii) environmental pathogens (Radostits et al., 2007). The contagious pathogens that are live on host's udder or skin causing intramammary infection, and spread as a reservoir from one animal to others within the herd through milkers or milking machine (Nickerson, 1994). Environmental mastitis in animals indicates poor

hygienic conditions of the shed as well as animals particularly in its udder (East et al., 1987; Oliver et al., 2011; Constable et al., 2017 and Horpiencharoen et al., 2019).

Different approaches and tools are used to detect mastitis in ruminants such as visual examination of udder and teats, macroscopic examination of milk, california mastitis test, direct microscopic somatic cell count, milk differential leukocytes analysis, ultrasonographical evaluation of udder and teats, and bacteriological culture (Adkins and Middleton, 2018 and Machado, 2018). California mastitis test is a simple, cheap, semiquantative and quick screening test for diagnosis of mastitis in ruminants (Schalm and Noorlander, 1957; Contreras et al., 1996; Smith and Sherman, 2009). Although the negative result of CMT is a good indicator of the absence of infections, the positive result of CMT may not be indicator of infectious process in udder of goats. Because the presence of epithelial cells in goat's milk are higher than cow's milk, which combine with leukocytes leading a different interpretation of CMT test not like as cattle (Lewter et al., 1984). Somatic cells in milk are mixed of both increase number of leukocytes and relatively small number of epithelial cells detaching from glandular secreting tissues. The concentration of somatic cells in goat milk is higher compare to cow and sheep milk due to apocrine secretion of goat's mammary gland (Contreras et al., 1997 and Paape et al., 2007). Direct microscopic somatic cell count (DMSCC) is the standard method determining somatic cell counts in milk of goats and cattle (ISO/IDF, 2008), whereas bacteriological culture is considered as a gold standard for detection of mastitis (Constable et al., 2017). Correa et al. (2010) has been suggested a threshold of SCC 1 million cells/ml of milk for identifying pathogens in goats and Petzer et al. (2017) suggested SCC below 200,000 cells/ml of milk is widely used as a threshold level to differentiate healthy gland from subclinically infected gland in cattle. Along with ultrasonography is a modern, rapid, accurate and non-invasive technique for investigation of physiological and pathological architectural changes of mammary gland in ruminants (Wojtowski et al., 2006; Slosarz et al., 2010; Tiwari et al., 2014). The benefits of this technique are easily portable and safe for both animal and operator because of free from radiation hazards. The ultrasonographical appearance of normal udder parenchyma as a homogenous, hypoechoic structure with anechoic rounded structures of lactiferous ducts and blood vessels (Tiwari et al., 2014; Fasulkov et al., 2014; Amin et al., 2017). The ultrasound images of teat structures allow an anechoic lumen surrounded by teat

wall comprising three distinct layers whereas teat canal is visualized as an anechoic lumen surrounded by two hyperechoic line, and teat orifice appears as a small anechoic area at the tip of the teat (Fasulkov et al., 2013, 2014; Tiwari et al., 2014; Adam et al., 2017; Amin et al., 2017; Barbagianni et al., 2017). Ultrasonography of mammary gland can be an applicable technique concurrent with commonly used diagnostic methods for evaluation of udder health. Additionally, hematological procedure has important diagnostic value that provides significant informations together with general examination of the patient (Kelly, 1984; Oyewale and Olowookorun, 1986). The health condition of an animal is truly reflected by accurate hematological examination of the animal (Ajuwape et al., 2005). Erythrocyte indices and leukocyte counts may be important predictors for accessing systemic tissue injury responses where cellular immune response in the blood may differ depending on the stage and existence of type of inflammation in body (Sordillo et al., 2009; Aitken et al., 2011).

Mastitis causes great economic loss in farmers through decrease milk production, cost of treatment and early culling of animals. Therefore, it is necessary to early and accurate detection of clinical entity and to recognize severity and prognosis of the disease which minimizes the economic loss. Considering the reason, clinical and ultrasonographical examination of mammary gland in ruminants were performed in this clinical study.

Objectives:

The present study was conducted to meet the following objectives:

1) To estimate direct microscopic somatic cell count in milk as an indicator of mastitis in goats and cattle.
2) To evaluate the udder and teats ultrasonographically during mastitis in goats and cattle.
3) To study the hematological alterations during mastitis in goats and cattle.

Chapter - 2: Review of Literature

2.1 Anatomy of the mammary gland

The caprine and bovine mammary gland is located in inguinal region. The mammary gland of goat and cattle is composed of two quarters and four quarters respectively which are divided by well demarcated inter-mammary groove. Each quarter comprises mammary body which included mammary parenchyma, ductal and cavity system and teat. There is no communication between the ductal systems of each quarters. The mammary parenchyma composed of secretory cells called alveoli where as several alveoli form a lobule and several lobules form a lobe. The ductal system comprises lactiferous ducts which are separated by elevated folds. The lactiferous ducts arise as holes, and irregularly open into a wide irregular pouch called glandular sinus or gland cistern (Adam et al., 2017; Amin et al., 2017). The wall of the glandular sinus comprises mucosal folds, and the gland cistern and teat cistern are separated by annular folds (Fasulkov et al., 2013).

Teat is the membranous tubular structure by which drain out of milk produced from secretory alveolar cells. The size and shape of teat are independent and variable of length, diameter and wall thickness (Nickerson, 1994). The teat wall is composed of three layers. The most outer layer is skin which has few number of fine hairs and hair follicles with clusters of sebaceous glands but absent in cattle. But the tip and middle part of teat are free from hairs in goats. The middle layer is fibro-musculo-vascular layer which is composed of dense connective tissues, circular smooth muscles and blood vessels. The most inner layer of teat cistern is mucosal epithelial lining consisting two layers of cuboidal cells. But the epithelial lining of teat canal and teat orifice are consisted of keratinized stratified sequamous epithelium which is resistance against infection (Mahdi, 2009). The sinus mucosal folds converage dorsal to the teat canal to form a rosette called Furstenberg rosette. It prevents microbial invasion from teat canal to teat sinus because it aggregates a large quantity of intraepithelial lymphocytes (Amin et al., 2017; Senthilkumar et al., 2020).

The anatomical criteria of udder like size and shape are important determinant of milk yield and milking ability of goats (James et al., 2009). Similarly, the anatomical and functional traits of teats like teat sinus, teat canal and teat end are great influence on

milk flow performance (Amin et al., 2017). The teat canal is the first line of defense against invasion of microorganisms into the udder parenchyma. Different sizes, shapes, placement of teat and morphology of teat end considerably indicate the probability of occurring mastitis (Bardakcioglu et al., 2011).
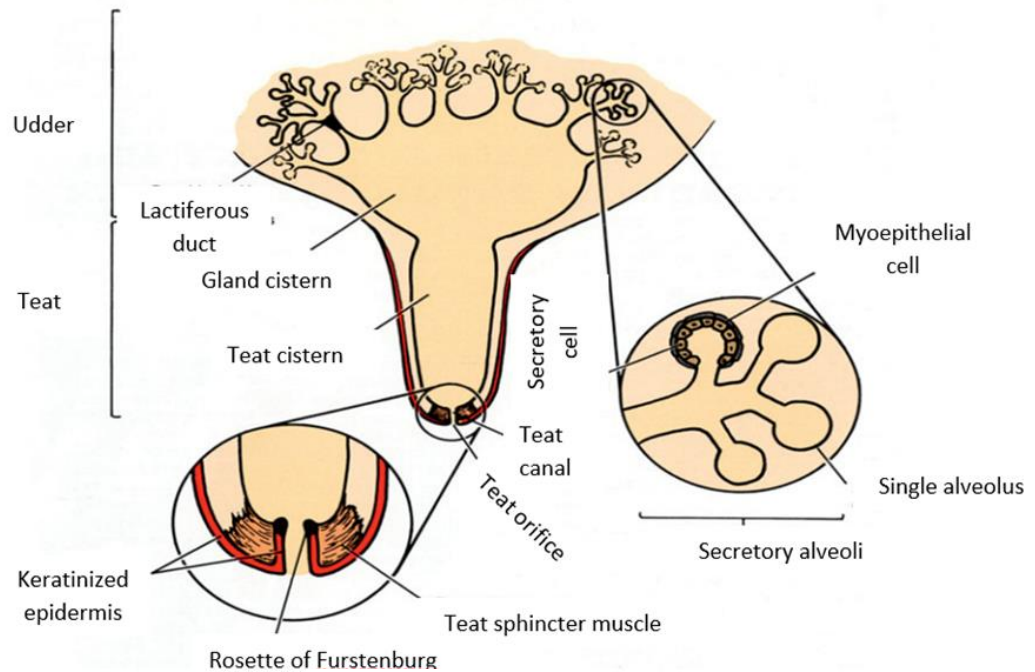
(Blowey and Edmondson, 2010)

Figure 2.1: Anatomical structures of the mammary gland in ruminants

2.2 Mastitis

Mastitis is the inflammation of mammary gland resulting from systemic hematogenous infection to udder or directly entry of pathogens through teat orifice to mammary parenchyma, where physical and/or chemical changes in milk composition and pathological alterations in glandular tissues (Radostits et al., 2007).

2.2.1 Classification of mastitis

Mastitis can be classified in different ways based on nature of transmission, persistence of signs and also based on presenting clinical signs. Depends on the presenting clinical signs in animal, mastitis can be classified two types: i) subclinical mastitis and ii) clinical mastitis. Different forms of clinical mastitis are seen in dairy animals such as per-acute gangrenous form, acute form and chronic form (Radostits et al., 2007).

2.2.1.1 Subclinical mastitis

Subclinical mastitis is characterized by alterations in chemical compositions of milk but there is no evidence of macroscopic changes i.e. signs of inflammations in mammary gland. Subclinical mastitis can be detected by different techniques such as California Mastitis test, direct or indirect counting of somatic cells in milk, using ultrasound technique and finally confirmed by microbial isolations (Machado, 2018).

2.2.1.2 Clinical mastitis

2.2.1.2.1 Acute mastitis

Acute clinical mastitis is characterized by presenting the signs of inflammation such as swelling, reddening, warming and touching to pain, and also visible changes in milk such flakes, clots etc. (Constable et al., 2017). The animal shows also others clinical signs such as anorexia, depression, fever and decreased milk production. There is also found enlargement of the retro-mammary lymph nodes and observe reluctant to move (Machado, 2018). In small ruminants, Claudication is a common clinical sign when they move their legs not to touch the inflamed udder (Kirk et al., 1996). The common way to detect acute mastitis is strip cup method where gross changes in milk are visible. Inspection and palpation of udder for signs of inflammation can also helpful to identify acute clinical mastitis. (Awad et al., 2008; Singh et al., 2018)

2.2.1.2.2 Chronic mastitis

Chronic mastitis is characterized by secretory tissues of udder parenchyma are gradually converted into fibrous tissues and a great loss of milk production. The size of fibrotic quarter is shrunken than normal quarter. On palpation, the variable sizes of abscesses may also be found which are hard in structures, painless and uneven surface. Abscesses are commonly found in caudal aspect of the affected quarter (Jackson and Cockcroft, 2002).

2.2.1.2.2 Per-acute gangrenous mastitis

Per-acute gangrenous mastitis is characterized by swollen, bluish discoloration, fluid exudation and coldness of affected mammary gland. The generalized clinical signs such as initially fever followed by hypothermia, depression, anorexia, dehydration are found in the affected animals. The animals become gradually weak and fall into recumbent conditions. Case fatality rates are high because of toxaemia if untreated. In

severe conditions, subcutaneous emphysema and crepitation sound also found in affected mammary gland (Menzies and Ramanoon, 2001; Abd-El-Hady, 2015). Goats are more frequently affected with gangrenous mastitis than cattle (Machado, 2018).

2.2.2 Etiology of mastitis

Mastitis is caused by many different species of infectious agents such as bacteria, fungi and virus (Menzies and Ramanoon, 2001). The pathogens causing mastitis are commonly classified into two groups: i) contagious pathogens and ii) environmental pathogens (Radostits et al., 2007).

2.2.2.1 Contagious pathogens

The pathogens that are live on host's udder or skin causing intramammary infection, and spread as a reservoir from one animal to others within the herd through milkers or milking machine (Nickerson, 2011). Goats are less commonly affected by contagious mastitis comparatively than cattle (Smith and Sherman, 2009). The common contagious pathogens in goats are Staphylococcus aureus, Corynebacterium bovis, Mycoplasma agalactiae, Mycoplasma mycoides var mycoides, Mycoplasma capricolum subsp capricolum and Streptococcus agalactiae (Bergonier et al., 1997; Smith and Sherman, 2009; Verbeke et al., 2014)

2.2.2.2 Environmental pathogens

The pathogens that are commonly found in environment of shed and acquire infection in udder from environment or through using contaminated utensils in the shed. Environmental mastitis indicates poor hygienic conditions of the shed as well as animal particularly udder. Coliform bacteria are most commonly cause environmental mastitis in dairy animals (Menzies and Ramanoon, 2001; Constable et al., 2017). The common environmental coliforms include Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Arcanobacterium pyogenes and Pseudomonas aeruginosa (East et al., 1987; Sumathi et al., 2008; Gao et al., 2017; Tomazi et al., 2012).

2.2.2.3 Miscellaneous pathogens

Miscellaneous pathogens are isolated from mastitic milk of goat and cattle including Yersinia pseudotuberculosis (Cappucci, 1978; Jones, 1982), Nocardia (Bassam and Hasso, 1997; Rozear et al., 1998), Corynebacterium spp. (Schaeren and Maurer, 2006; Hall and Rycroft, 2007), Listeria monocytogenes (Sasshofer et al., 1987), Mannheimia haemolytica, and Actinobacillus equuli (Ameh et al., 1993). Fungi and

yeast are also isolated from mastitic animals like Cryptococcus neoformans (Pal and Randhawa, 1976; Aljaburi and Kalra, 1983). Lentiviruses also cause mastitis in small ruminants, and significantly changes in chemical composition of milk in affected animals (Menzies and Ramanoon, 2001; Junior et al., 2007)

2.3 Epidemiology

2.3.1 Prevalence

The prevalence of clinical mastitis in goat is recorded 5.273% under rural condition in Bangladesh (Sarker and Samad, 2011). According to Contreras et al. (2007), the prevalence of clinical mastitis is less than 5% in goat. The prevalence of subclinical mastitis in goat is recorded 50.9% based on CMT test under Chattogram metropolitan city in Bangladesh. In general, the prevalence of SCM in goat is ranged between 20 to 50% (Bergonier et al., 2003). The Incidence of clinical mastitis in cow ranges from 10-12 % per year where the prevalence of intra mammary infection is about 50% of cows and 10-25 % of quarters (Radostits et al., 2007). The prevalence of subclinical mastitis in dairy cattle is 33.56% based on CMT in in Chittagong district of Bangladesh (Barua et al., 2014).

2.3.2 Animal level risk factors

2.3.2.1 Breed

Black Bengal goat, Jamnapari, non-descriptive indigenous goat and cross breed are commonly found in Bangladesh (Akter et al., 2020). Jamnapari breed are more susceptible to mastitis than the Black Bengal goats and cross breeds. The higher odds ratio of SCM is found in amnapari breed than Black Bengal goats and cross breeds (Akter et al., 2020). *Bos taurus* taurus (exotic breeds in Indian sub-continent and Africa are six times more associated with CM than *Bos Taurus* indicus (native breeds in Indian sub-continent and Africa) (Oliveira et al., 2015). Zebu cattle was frequently reported to show less susceptibility to CM probably due to low genetic potential for milk production (Wilson et al., 1997; Shem et al., 2002; Dego and Tareke, 2003). Holstein Friesian is an exotic breed and this breed has high genetic potentiality of dairy characters which has been proved associated with susceptibility to CM (Hansen et al., 2002).

2.3.2.2 Body condition score

Goats with low BCS are susceptible to mastitis than does with moderate BCS (Megersa et al., 2010). The higher odds of SCM is found in goats with good BCS than goats with poor BCS (Akter et al., 2020). Poor BCS was associated with a higher incidence rate of mastitis 43.1 cases per 100 cow per year at risk compared to the group of fair (37.2 cases per 100 cows per year) and good BCS (33.5 cases per 100 cows per year) of lactating cows (Kivaria et al., 2007).

2.3.2.3 Age

Age of animal is an important risk factor for prevalence of mastitis in goat (Boscos et al., 1996; Sharma et al., 2007; Ali et al., 2010). According to (Ferdous et al., 2018), the prevalence of mastitis is higher at 4-5 years of age than 2-3 years of age. The advanced age is epidemiologically associated with subclinical mastitis in goat. The prevalence of mastitis in cattle is gradually increased with age highest at 7 years of old (Radostits et al., 2007). Barua et al. (2014) reported that the age of 9-18 years was higher prevalence (45.65%) of mastitis in cattle than the age of 3-8 years (38.07%).

2.3.2.4 Parity

Parity is considered as a risk factor for intramammary infection in goat (Sánchez et al., 1996). According to Ferdous et al. (2018), the prevalence of mastitis in goat is higher in 5th parity than 2nd parity. The incidence of subclinical mastitis is higher in multiparous goats than primiparous goats (Bergonier et al., 2003). The prevalence of mastitis is more in goats having 3 or more kids than goats having 1 or 2 kids (Ferdous et al., 2018). In cattle, parity-wise mastitis shows 35.8, 56.7 and 76.7 cases per 100 cows per year at risk correspondingly for parity of 1-4, 5-8 and ≥ 9th parity (Kivaria et al., 2007). In another study, animals of 3rd parity or more and, 2nd parity showed a higher tendency for having CM compared to newly calved heifers (Breen et al., 2009). Barua et al. (2014) reported that the 4 to rest parity was higher prevalence (48.98%) of mastitis in cattle than parity of 1 to 3 (37.50%).

2.3.2.5 Lactation stage

The prevalence of mastitis is higher in early period of lactation than late lactation stage (Las Heras et al., 1999; Leitner et al., 2001). The odds ratio of SCM is higher in late lactation stage compared to early stage of lactation (Persson et al., 2014; Stuhr et

al., 2013). In cattle, the prevalence of SCM in late lactation (72.45%) was greater than the early lactation (40%) and mid lactation period (27.56%) (Islam et al., 2010).

2.3.3 Quarter level risk factors

The prevalence of both subclinical and clinical mastitis is higher in right quarter than left quarter (Ferdous et al., 2018). Goats with globular shaped udder are found lower CMT value compared to non-globular shaped udder in goats (Montaldo and Martinez-Lozano, 1993). Goats having non-ballon shaped teats are found lower CMT value than goats having ballon shaped teats (Montaldo and Martinez-Lozano, 1993; Margatho et al., 2020).

2.4 Diagnosis of mastitis

Different approaches or techniques and tools are used to detect mastitis in dairy animals such as visual examination of udder and teats, macroscopic examination of milk, California Mastitis test, direct somatic cell count, somatic cell analysis and ultrasonography.

2.4.1 Gross examination of the udder and teat

The diagnosis of mastitis begins with visual inspection of udder and teat observing any alteration of color such as reddening, bluish etc., any changes in size like as induration or atrophy, and any changes in shape like as asymmetry (Smith and Sherman, 2009). Gently palpate and rolling of the teat between thumb and other fingers to determine presence of any pain and obstruction (Steiner, 2004). The Udder also gently palpate to detect presence of any abnormities such as diffuse nodules, harden consistency in parenchyma and increase local temperature of udder. The supramammary lymph nodes are also palpated to determine enlargement and consistency (Machado, 2018).

2.4.2 Macroscopic examination of milk

The visual inspection of the milk is performed by using a strip cup that has a black background or screened cup for detection of macroscopic changes in milk such as discoloration like serous or blood stained as well as flakes, clots or purulent materials. Milk from affected quarter is drawn on to the black plate, and is compared with the milk of healthy quarter (Radostits et al., 2007).

2.4.3 California Mastitis Test

California mastitis test is a subjective, simple, inexpensive, more useful, semiquantitative and quick screening test for diagnosis of mastitis (Schalm and Noorlander, 1957; Contreras et al., 1996; Machado, 2018; Smith and Sherman, 2009). It determines the numbers of somatic cells (both leucocytes and epithelial cells) present in milk. It is an anionic solution of detergent containing 3% alkyl arylsulfonate with bromcresol purple as a pH indicator showing violet coloration in case of positive (alkaline) samples. The detergent reacts on the both leucocytes and epithelial cells releasing genetic materials of the cells, and accelerates viscosity which is directly proportional to the number of cells present in milk (Watson and Buswell, 1984; Peixoto et al., 2012; Machado, 2018). Although the negative result of CMT is a good indicator of the absence of infections, the positive result of CMT may not be indicator of infectious process in udder. Because the presence of epithelial cells in goat's milk are higher than cow's milk, which combine with leukocytes leading a different interpretation of CMT test not like as cattle (Lewter et al., 1984). The collection of milk sample is performed before milking and immediately after discarding the first milk jets, and equal quantities (typically 2 ml) of CMT reagent and milk are added into CMT paddle (Smith and Sherman, 2009). Different countries of the world are used different scoring system of the CMT test. According to the Schalm et al. (1971), the score of CMT: 0 (negative or no reaction), T (Trace) (slight slime), 1 (distinct slime but without gel), 2 (Immediate gel formation moving as a mass during swirling), 3 (gel develops a convex surface and adheres to the bottom of the cup). On the other hand, in Scandinavian system, there are five categories of CMT scores of 1 to 5 which are depending on the amount of gel formation and color deepness. According to Silva et al. (2001), the reactions of CMT test scores as 0: negative (no reaction between reagent and milk), 1: traits (suspected), 2: weakly positive reaction, 3: positive reaction and 4: strongly positive reaction.

2.4.4 Somatic cell count of milk

Somatic cells in milk are mixed of both large number of leukocytes and relatively small number of epithelial cells detaching from glandular secreting tissues. These cells play a vital role in natural defense mechanism of mammary gland of animals (Shearer and Harris, 2003). In the presence of any injury or infection of mammary gland, the significant number of somatic cells accumulate in milk (Raynal-Ljutovac et

al., 2007). The concentration of somatic cells in goat milk is higher compare to cow and sheep milk (Contreras et al., 1997; Paape et al., 2007). The secretion of milk in goat is apocrine in nature leading higher amount of cytoplasmic particles in milk compared with other species (Dulin et al., 1983; Paape and Capuco, 1997; Souza et al., 2012). To establish a threshold cell count in goat milk is difficult for diagnosis of mastitis because many authors have used different technique for somatic cells enumeration in milk (Smith and Sherman, 2009). Poutrel and Lerondelle (1983) have been suggested a threshold of 1 million cells/ml of milk for identifying major pathogens in early and middle lactation in goat. On the other hand, a threshold at 750×10^3 cells/ml and 1750×10^3 cells/ml of milk for minor and major pathogens respectively in goat (De Crémoux et al., 1996). However, the standard of somatic cells count in goat milk is not more than 1.0 million cells/ml of milk in United States (Shearer and Harris, 2003). For counting the somatic cells in goat milk accurately, only DNA specific methods should be used (Dulin et al., 1983; Sierra et al., 1999; Marco et al., 2012). In lactating mammary gland of healthy cow, the SCC is less than 1×10^5 cells/mL of milk but during intramammary infection, the glandular scc can increase to more than 1×10^5 cells/mL of milk (Radostits et al., 2007). Petzer et al. (2017) stated that SCC thresholds of greater than 2×10^5 cells/mL were used in quarter levels for detection of subclinical mastitis in cattle. Direct microscopic somatic cell count (DMSCC) is the standard method determining somatic cell counts in milk for dairy animals (ISO/IDF, 2008). The DNA specific stains like pyronin Y-methyl green, MayGrünwald-Giemsa and Gallego's trichrome are most frequently used in DMSCC method (Gonzalo et al., 1998; Berry and Broughan, 2007).

2.4.5 Milk Differential Leukocyte Count

Somatic cells consist of leukocytes and epithelial cells, and these cells obtain in healthy mammary gland of dairy animals. The leukocytes include polymorphonuclear leukocytes (PMNLs) mainly neutrophils, macrophages and lymphocytes whereas neutrophils play a vital role against pathogens, and consider the first line of immunological defense for this reason goats are more resistant to mastitis (Tian et al., 2005). After invading of any pathogens in mammary gland, these cells stimulate inflammatory process by releasing chemo-attractants (Paape and Capuco, 1997; Paape et al., 2002; 2003; Rainard and Riollet, 2003). The chemical substances rapidly increase influx of polymorphonuclear leukocytes, particularly neutrophils into site of

infection from blood by chemotaxis and diapedesis (Paape and Capuco, 1997; Gonzalo et al., 1998; Paape et al., 2007; Albenzio and Caroprese, 2011) and comprise over 90% of somatic cells in milk of affected udder (Morgante et al., 1996; Cuccuru et al., 1997).

Differential somatic cells in milk from healthy mammary gland of dairy animals comprises PMNLs (40-87%), macrophages (15-41%), lymphocytes (4-20%), eosinophils and epithelial cells present as lower levels (Paape and Capuco, 1997; Winnicka et al., 1999; Menzies and Ramanoon, 2001; Paape et al., 2001; Haenlein, 2002; Bergonier et al., 2003; Raynal-Ljutovac et al., 2007). According to Boulaaba et al. (2011), PMNLs, macrophages and lymphocytes comprise 79.2% ±11.5%, 17.8 ±10.2% and 2.8±3.6% of the cells respectively by flow cytometry in healthy udder. Besides, by using direct microscopic counting method specific for DNA to identify the percentage of PMNLs, macrophages and lymphocytes are 80.9 ± 9.3%, 15.0 ± 7.7% and 4.2 ± 3.9% respectively. However, the percentage of PMNLs (85.5 ± 6.0%) is higher and the percentages of macrophages (13.0 ± 5.6%) and lymphocytes (1.4 ± 0.9%) are lower compare with healthy ones (Boulaaba et al., 2011). Differential cell count may be used to distinguish physiological variations of cells from pathological cellular variations leading to detect intra-mammary infections (Cuccuru et al., 1997).

2.4.5 Ultrasonography of the mammary gland

Ultrasonography is a rapid, accurate and non-invasive technique for investigation of physiological and pathological architectural changes of mammary gland in ruminants (Wojtowski et al., 2006; Slosarz et al., 2010; Tiwari et al., 2014). The diagnostic imaging technique relies on sound reflection between the closely related structures producing echo in term of hyperechoic, hypoechoic and anechoic for characterization of morphological structures like mammary gland (Descoteaux et al., 2010). The ultrasound examination of udder parenchyma in small ruminants is performed in standing condition through direct contact method applying acoustic coupling gel. In dairy animals, the udder parenchyma is scanned horizontally using B-mode ultrasound with linear, sector or convex transducer (5-7.5 MHz) (Ruberte et al., 1994; Santos et al., 2014; Adam et al., 2017). In ruminants, teat morphology is scanned vertically using different tecniques like "direct contact", "stand-off" and "water bath" techniques with 5, 7.5 and 10 MHz frequency linear-array transducers (Fasulkov et al., 2013). The "water bath" technique along with 10 MHz frequency linear transducer

provides the best possibility to visualize all teat structures like teat orifice, teat canal, rosette of Furstenberg, teat wall, teat cistern and the boundary between teat and gland cisterns (Fasulkov et al., 2013).

The parenchyma of normal mammary gland appears as a homogenous, hypoechoic structure with anechoic rounded structures of lactiferous ducts and blood vessels (Tiwari et al., 2014; Fasulkov et al., 2014; Amin et al., 2017). The gland sinus or cistern appears as anechoic space and the wall of the gland sinus appear mixed of hyper to hypoechoic area (Tiwari et al., 2014; Adam et al., 2017). The annular fold appears as a linear hypoechoic structure between the gland sinus and teat cistern or sinus (Adam et al., 2017). The ultrasound image of teat structures allow an anechoic lumen surrounded by teat wall comprising three distinct layers; outer layer (skin) appears as hyperechoic, middle layer (muscle and connective tisssues) appears as hypoechoic and inner layer (mucosa) allows as a hyperechoic line (Fasulkov et al., 2013, 2014; Tiwari et al., 2014; Adam et al., 2017; Amin et al., 2017; Barbagianni et al., 2017). The rosette of Furstenberg appears as a short hyperechoic structure between teal canal and teat sinus (Motomura et al., 1994; Nak et al., 2005; Khol et al, 2006; Franz et al., 2009; Rambabu et al., 2009; Szencziova and Strapak, 2012; Amin et al., 2017). The teat canal is visualized as an anechoic lumen surrounded by two hyperechoic line and teat orifice appears as a small anechoic area at the tip of the teat (Fasulkov et al., 2013; Tiwari et al., 2014; Adam et al., 2017). Ultrasonogrically, the supramammary lymphnode of health mammary gland represent well demarcated thin capsule with hypoechoic cortical area and central hyperechoic hilus (Bruneton et al., 1994; Hussein et al., 2015; Abd Al-Galil and Khalil, 2016).

The image of udder parenchyma during acute mastitis in ultrasound is characterized by a non-homogenous and hypo to hyperechoic structure associated with inability to clear visualization of anechoic alveoli, lactiferous ducts and blood vessels (Fasulkov et al., 2014, 2015; Amin et al., 2017). The teat walls become thicken and hyperechoic and teat sinus is filled with numerous hyperechoic structure like milk clots, flakes etc., and not clearly visualize Furstenberg rosette and teat canal (Awad et al., 2008; Fasulkov et al., 2013, 2014). The significant changes are found in internal structure of supramammary lymph nodes (Bradley et al., 2001, Amin et al., 2017). In case of subclinical mastitis, the mammary parenchyma shows homogenous hypoechoic structure but lack of clear visualize of alveoli and lactiferous ducts, and

hypoechogenic contents appear in gland cistern (Hussein et al., 2015; Abd Al-Galil and Khalil, 2016). The teat walls become slightly thicken and irregular contour lining (Abd Al-Galil and Khalil, 2016). The supramammary lymphnode becomes enlarge and complete hypoechoic structure (Bruneton et al., 1994; Hussein et al., 2015; Abd Al-Galil and Khalil, 2016). Gangrenous mastitis in goat, the udder parenchyma remains more heteroechogenic contented fluids due to excessive debris and shows focal hyperechogenic areas (Awad et al., 2008; Amin et al., 2017). In chronic mastitis, the glandular parenchyma shows more hyperechoic areas and few lactiferous ducts and fibrotic hyperechoic changes found in teat wall (Flock and Winter, 2006; Amin et al., 2017).

2.5 Haematological alterations during mastitis

The Hematological procedure has important diagnostic value that provides significant informations together with general examination of the patient (Kelly, 1974; Oyewale and Olowookorun, 1986). The health condition of an animal is truly reflected by accurate hematological examination of the animal (Ajuwape et al., 2005). The packed cell volume (PCV), Total erythrocyte counts and hemoglobin (Hb) concentration are higher in goats and cattle compare to non-mastitis animals (Ajuwape et al., 2005; Abba et al., 2013; Hristov et al., 2018). The total leukocyte counts (TLC) are increase significantly in animals affected mastitis compare to healthy lactating goats (Ajuwape et al., 2005; Abba et al., 2013; Hussien et al., 2015). The differential leukocyte counts include percentage of neutrophils, lymphocytes, monocytes, eosinophils and basophils. The percentage of neutrophils increase in mastitic animals relative to normal lactating goats whereas the percentages of lymphocytes, monocytes and eosinophils are decrease (Abba et al., 2013; Hussien et al., 2015). According to Ajuwape et al. (2005), the neutrophilia, lymphocytosis, monocytosis and eosinophilia are found in mastitis affected goats due to inflammatory response resulting from acute tissue necrosis and endotoxin released from bacteria.

2.6 Treatment strategy of mastitis

National Mastitis Council (NMC, 2017) recommends that dairy animals with CM not be treated with antibiotics until the results of bacteriological culture results are available to determine the type of organisms. The samples will be tested on laboratory, and the culture results will be forwarded and antibiotics will be chosen accordingly within 24 hours. Animals infected with Gram negative bacteria (E. coli or

Klebsiella spp.) will not be given antibiotics, but will be given supportive drugs such as oxytocin to improve milk let-down, intravenous or oral fluid, and non-steroid anti-inflammatory drugs. Antibiotics will be prescribed for Gram-positive bacterial infections based on the results of antibiotic susceptibility testing. During this period of culturing (except for severe CM), animals will only be treated with supportive therapy such as oral or systemic fluid and anti-inflammatory drugs if the animal has dehydration or fever, depending on the severity of clinical symptoms (Adkins and Middleton, 2018). In Bangladesh, there is currently no established guideline for treating CM cases. This is a major focus of current research, as an ideal treatment strategy for CM cases will be implemented through knowledge of correct prognosis detecting of pathological alterations in mammary gland using ultrasound.

2.7 Prevention and control of mastitis

There are predefined 10-point control strategies by National Mastitis Council, (2017) for controlling the occurrence of mastitis: i) Udder health goal should be established by focusing on periodic SCC and tracking progress over time. ii) Stall size should be adequate, and a clean and comfortable environment should be maintained. iii) Standard milking procedures must be followed, ensuring pre and post-dipping of teats and hand disinfection before milking. iv) Installation, servicing, and replacement of improper milking equipment should be done based on examination v)) Good record keeping should be ensured, vi) Proper CM management will be considered during lactation, vii) Effective DCT through proper feeding plan, drying the udder, sealing of the teats with long acting antibiotics, viii) Strict biosecurity maintenance using the BMSCC data for 21 suspicion and diagnostics, aseptic milk sampling, segregation or culling of infected animals based on bacteriological confirmation, ix) Ordinary observing of udder wellbeing status through enlistment in customary SCC checking program, checking the variety in dispersion of SCC values, computing the high SCC and CM occurrence rates for going with treatment and advertising choices, and x) At long last, occasional audit of complete mastitis control program will be kept up with by a warning group including veterinarian, maker, crowd director and draining staff (National Mastitis Control, 2017).

Chapter - 3: Materials and Methods

3.1 Area and period of study

The study was conducted at Shahidul Alam Quaderi Teaching Veterinary Hospital (SAQTVH) of Chattogram Veterinary and Animal Sciences University (CVSAU), Chattogram, Bangladesh during the period of January 2022 to September 2022.
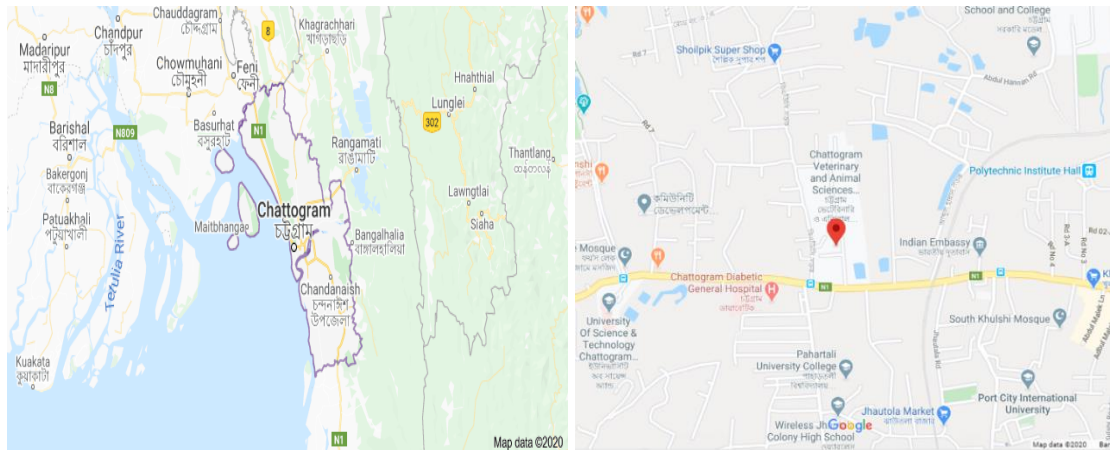
Figure 3.1: Geographical location of the study area

3.2 Criteria of study population

The study was carried out on lactating crossbred goats of Jamnapari, where lactation period was 7 to 90 days, and lactating crossbred cattle of Holstein Friesian, where lactation period was 7 to 180 days. Any other disease conditions of the selected populations except normal and affected animals with mastitis were excluded from this study.

3.3 Study design

40 lactating crossbred Jamnappari goats and 10 lactating crossbred Holstein Friesian cattle were presented to SAQTVH forming the material of this study.
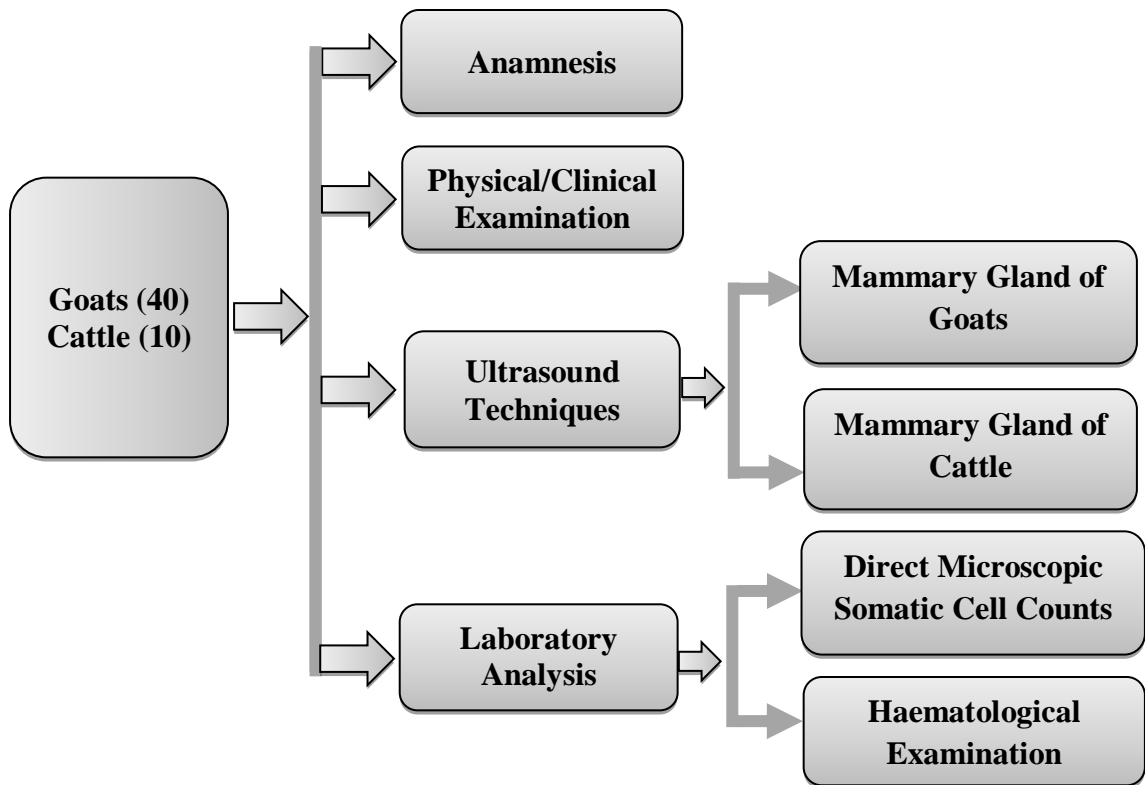
Figure 3.2: The study design in a schematic form

3.4 Data collection

A structured questionnaire was prepared and used to collect epidemiological data. The data related to animal level such as age, calving interval, parity, lactation stage, litter size were obtained by interviewing of the animal's owner. The structured questionnaire was given in appendix I.

Figure 3.3: Collection of epidemiological data from animal owners in hospital

3.4.1 Clinical examination of the goats and cattle

Heart rate (beats/min), respiration rate (breaths/min), rectal temperature (°F), general body condition (alert/dull/depressed/other), dehydration (mild/moderate/severe), besides other clinical signs exhibited by the animals were recorded. Clinical examination of mammary gland was conducted according to Baumgartner (1999) method. Udder shape (pendulous, spherical) and symmetry; teat shape (funnel, cylindrical and bottle) and symmetry were assayed accordance by Franz et al. (2009) and James et al. (2009). The skin condition of the udder and teats like erythema, bluish discoloration etc. were recorded. The udders were palpated for determining the consistency of the glandular tissues, and teats were palpated for determining the any hardness and obstruction in the area of the teat cistern and teat canal. Finally, the supramammary lymph nodes were examined for any enlargement of the lymph nodes in animals. All milk samples were examined physically, and the abnormalities of milk samples such as clotted milk, watery milk etc. were recorded.
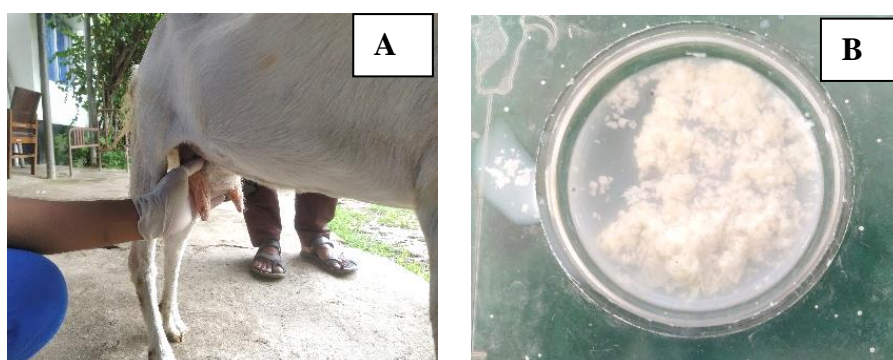
Figure 3.4: A) Clinical examination of udder, B) Physical examination of milk

3.4.3 Ultrasonographical examination

3.4.3.1 Instrumentation

In ultrasonographic studies, a portable, two-dimensional real time B-mode ultrasound machine (Exago, France) was used for scanning and equipped with 5-10 MHz linear transducer. For examination of teat, a transparent cylindrical plastic recipient was used which was filled with sterile water at 30-35^{0}C. The sterile water was changed after examination of a single animal. The udder and teats were cleaned thoroughly using 70% alcohol before scanning. For improving image quality, sterile ultrasound

coupling gel was used between udder's skin and probe in direct contact method for udder, and was also used between the plastic recipient and probe in water bath technique for teats.
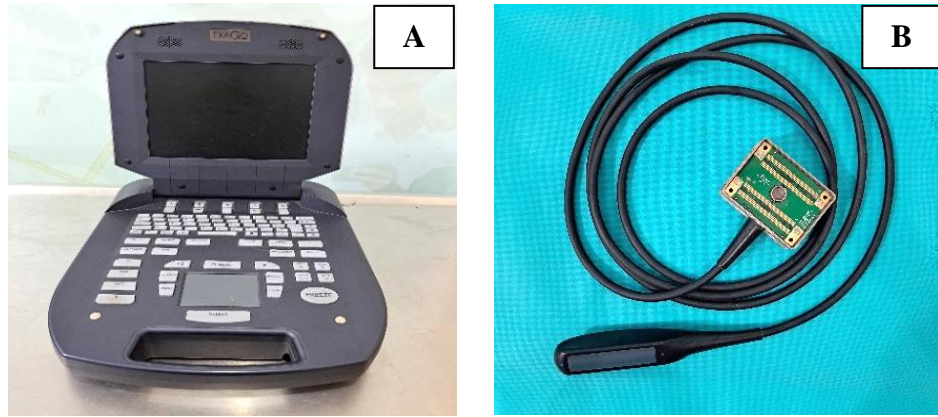
Figure 3.5: A) Portable ultrasound machine, B) 5-10 MHz linear probe

3.4.3.2 Preparation of the mammary gland for ultrasonography

For examination of mammary gland of goats, scanning was performed in standing condition after proper handling of animals. The scanning site of udder was clipped carefully for removing of fine small amount of hairs, and was cleaned with 70% solution of alcohol. The hairs of teats were also clipped and cleaned following the same procedures. For examination of mammary gland of cattle, scanning was performed in standing condition after proper restraining of animals with or without tranquilization.

3.4.3.3 Ultrasonographical examination of the udder

The scanning of udder parenchyma in animals were performed applying sterile ultrasound coupling gel with 5-10 MHz linear probe by direct contact method (contact between the transducer and the udder skin). For examine the whole udder, the transducer was placed on the caudal surface of each mammary gland along its longitudinal axis and moved upward and downward; and also repeated at the lateral surface of the mammary gland (Flock and Winter, 2006). The scanning depth was used 80 mm during the examination. For scanning of gland cistern, the probe was placed with 80^0 angle cranially just above the insertion (Ayadi et al., 2003). The same procedure was also followed for all quarters. The ultrasonographic appearance of the

udder parenchyma and its internal structures of normal (non-mastitis) and animals affected with mastitis were recorded.
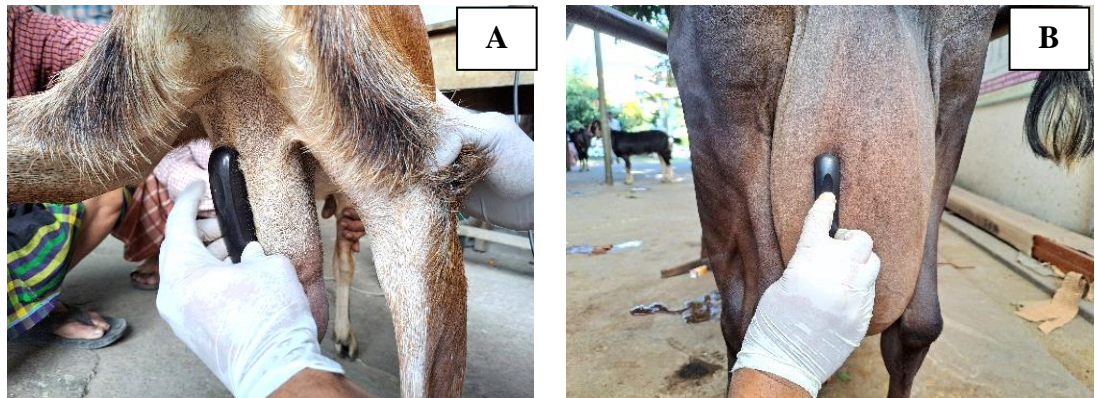
Figure 3.6: Ultrasonographical examination of the udder parenchyma with linear probe: A) goat, B) cattle

3.4.3.4 Ultrasonographical examination of the teat

Scanning of the teat in animals was performed by water bath method where the teat was immersed in a sterile water ($30\text{-}35^0$C) filled transparent plastic cup. A 5-10 MHz linear probe was used and the depth was remained 40 mm (Amin et al., 2017). The transducer was placed in a vertical plane that was parallel to longitudinal axis of the teat. A sterile ultrasound coupling gel was also applied between plastic cup and transducer to get good quality images. The probe was moved longitudinal axis of the teat beginning from its base to the teat orifice, in order to image the teat cistern and teat canal. The ultrasonographic appearance of the teat in normal animals and affected animals were recorded. The teat structures i.e. teat wall thickness, teat cistern diameter, teat canal length and teat canal diameter were measured in normal and affected animals.

Figure 3.7: Ultrasonographical examination of teat by water bath technique: A) goat, B) cattle

3.4.3.5 Ultrasonographical examination of supramammary lymph nodes

The scanning of supramammary lymph nodes were performed placing the transducer on the dorsal and lateral to the caudal aspect of the udder halves (Hussein et al., 2015). The ultrasonographic appearance and measurement of supramammary lymph nodes of normal and affected animals were recorded.

Figure 3.8: Ultrasonographical examination of the lymph node at base of the udder: A) goat, B) cattle

3.4.3.5. Milk sample collection, preservation and transportation

Milk samples were collected directly from each quarters of selected animals. Before collection, teats were cleaned with 70% ethanol and the first strip of milk was discarded. About 10 ml of milk samples were taken from each halves and were labeled on sterile falcon tubes. After collection, the milk samples were immediately transported using insulated ice box to the laboratory at CVASU. The milk samples were stored at 4°C for counting of somatic cells of milk.

Figure 3.9: Collection of milk sample from goat

3.4.3.6. Blood sample collection, transportation and preservation

Blood samples were collected from selected animal in accordance with Shaikat et al. (2013). Approximately, 2ml of blood was drawn aseptically from jugular vein of each animal using disposable sterile syringe and needle. The collected blood samples were transferred into a sterile vacutainer containing disodium ethylene diamine tetra-acetic acid (EDTA) (1mg/ml of blood). The sample containing vacutainers were labelled and transported in an ice box to CVASU laboratory for further haematological analysis.

Figure 3.10: Collection of blood samples from jugular vein: A) goat, B) cattle

3.4.4 Laboratory analysis

3.4.4.1 Direct microscopic somatic cell count

Somatic cell count of each milk sample was estimated in duplicate within 6 hours after collection. The milk sample was heated at 40°C in a hot water bath and was kept for 15 minutes at that temperature before being cooled to 20°C with stirring carefully. Accurately, 0.01ml of milk sample was taken using micropipette and placed on a $1cm^2$ (5mm × 20mm) defined area of degreased microscopic slide. The taken sample was smeared uniformly with sterile bacteriological plastic loop and the smears were air dried in a horizontal position. After drying overnight, the duplicate prepared smears were fixed by pouring few drops 96% ethyl alcohol on it for 3 minutes and air dried. Then fixed smears were defatted with xylol for 8 minutes and smoothly rinsed with 60% ethyl alcohol and again air dried. According Gonzalo et al. (1998), the smears were stained with to May-Grünwald Giemsa dye for 2 minutes, at 50% for 2 minutes and Giemsa solution for 20 minutes, and air dried. The smears were dehydrated in an increasing series of alcohols and xylols. According to ISO (2008), the somatic cell counts were measured under a microscope with 40X magnification and counted in 50 microscopic fields. The formula of counting of somatic cells by DMSCC method was given in appendix II. The study populations (goats and cattle)

were classified based on counting of DMSCC of milk samples which were given below:
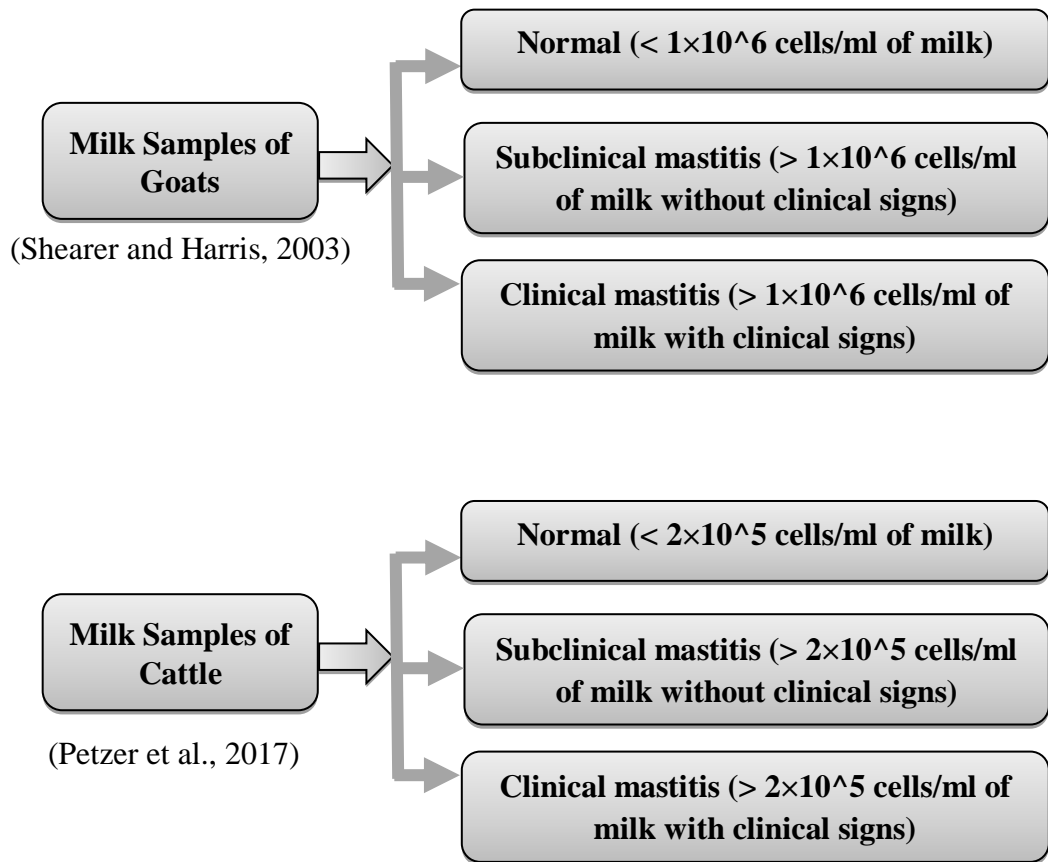
Figure 3.11: Diagnosis of mastitis based on DMSCC in schematic form
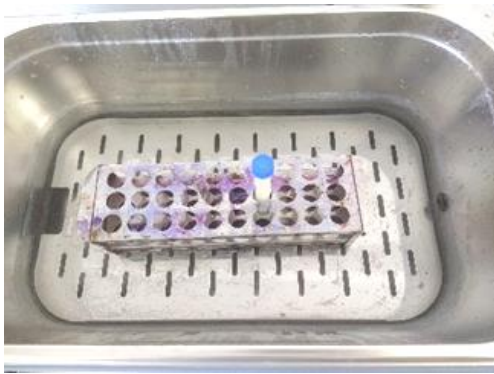
Figure 3.12: Milk sample was heated at 40°C at 15 min.
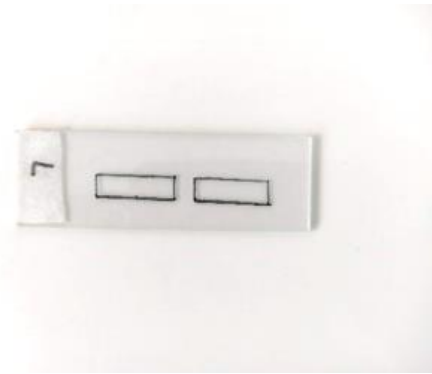
Figure 3.13: 1cm^2 (5mm $\times$ 20mm) defined area was drawn on microscopic slide

Figure 3.14: Milk sample was placed on slide to make smear

Figure 3.15: 0.01ml of milk sample was taken using micropipette
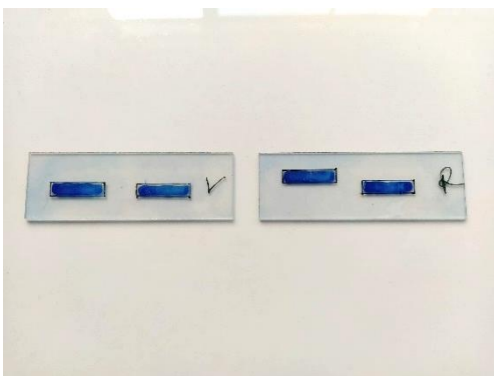
Figure 3.16: Smears were stained with May-Grünwald Giemsa
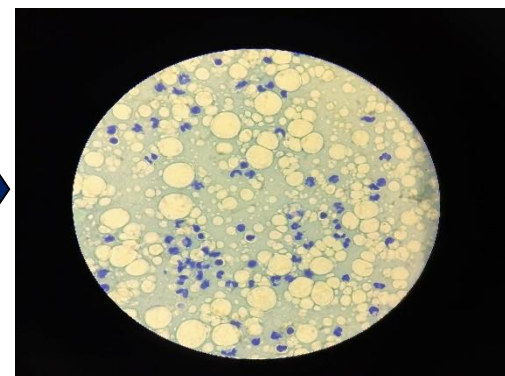
Figure 3.17: Somatic cells of milk were counted under microscope

3.4.4.2 Haematological examination

Total erythrocyte count (TEC) and total leukocyte count (DLC) were determined by counting red blood cells (RBC) and white blood cells (WBC) respectively using hemocytometer under a microscope. Packed cell volume (PCV) was measured by hematocrit method and concentration of hemoglobin (Hb) was determined by Sahli's method. For determining blood differential leukocyte counts (DLC), blood smears were prepared on clean glass slides and stained with Wright's stain. The stained blood smears were examined with oil immersion under microscope for leukocytes identification. The leukocytes were counted (100 cells) and classified; and determined the percentages of different leukocytes. The estimate routine blood parameters were measured according to the procedure published by Sharma and Singh (2000).

Figure 3.18: Measurement of haemoglobin by Sahli's hemoglobinometer.

Figure 3.19: Measurement of packed cell volume by hematocrit tube
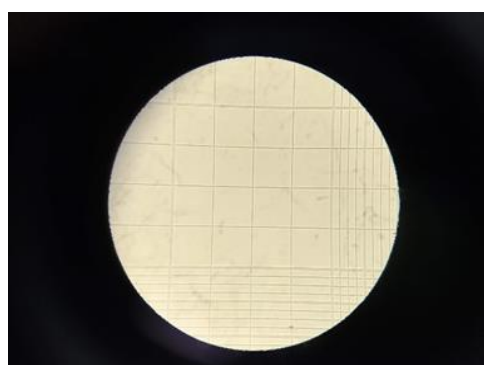
Figure 3.20: RBC count under microscope
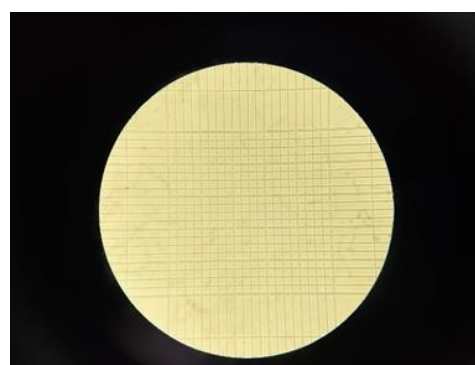
Figure 3.21: WBC count under microscope
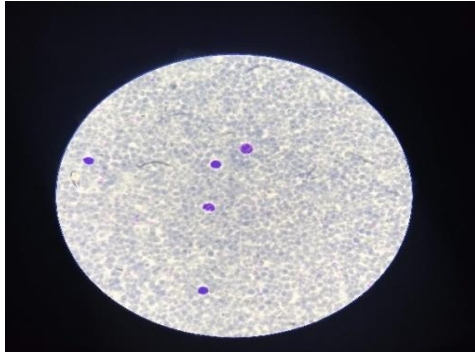
Figure 3.22: Differential leukocytes count under microscope
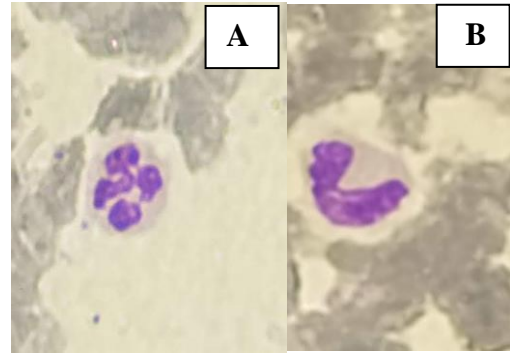
Figure 3.23: A) Mature neutrophil, B) band cell under microscope

3.5 Statistical analysis

The animals were assigned to normal, subclinical mastitis and clinical mastitis for convenience of analysis of results. For data entry, specially created data sheets were used in the "Microsoft Excel, Windows Version 16". The statistical evaluation was done with Minitab® 20 Statistical Software. The data were statistically analyzed using percentage and one-way ANOVA at 5% level of significance, and using the mean as hypothetical value. Tukey's test was performed to identify any significant difference in between two groups. The Chi-square test was performed to analyze the categorical data at 5% level of significance.

Chapter - 4: Results

The study population comprised 50 lactating ruminants including 40 goats and 10 cattle. In this study, all animals were categorized into three groups comprising healthy, subclinical and clinical mastitis. In goats, 17 animals (17/40, 42%) healthy and 13 animals (13/40, 32%) had subclinical mastitis and 10 animals (10/40, 25%) had clinical mastitis. The quarter levels in goats, 55% (22/40) right quarters were normal, whereas 27% (11/40) and 17% (7/40) were affected with subclinical and clinical mastitis respectively. On the other hand, 45% (18/40) left quarters were normal while 35% (14/40) and 20% (8/40) were affected with subclinical and clinical mastitis respectively (Table 4.1).

In cattle, 2 animals (2/10, 20%) had normal mammary glands, 3 animals (3/10, 30%) had subclinical mastitis and 5 animals (5/10, 50%) had clinical mastitis. The quarters level in cattle, 30% (6/20) fore quarters were normal, whereas 35% (7/20) and 35% (7/20) were affected with subclinical and clinical mastitis respectively. On the other hand, 20% (4/20) rear quarters were normal while 60% (12/20) and 20% (4/20) were affected with subclinical and clinical mastitis respectively. In individual quarter level, 30% (3/10) right fore quarter and 20% (2/10) right rear quarter were normal whereas 30% (3/10) right fore quarter and 50% (5/10) right rear quarter were affected with subclinical mastitis, and 40% (4/10) right fore quarter and 30% (3/10) right rear quarter were affected with clinical mastitis. On the other hand, 30% (3/10) left fore quarter and 20% (2/10) left rear quarter were normal whereas 40% (4/10) left fore quarter and 70% (7/10) left rear quarter were affected with subclinical mastitis, and 30% (3/10) left fore quarter and 10% (1/10) left rear quarter were affected with clinical mastitis (Table 4.1).

Table 4.1: The percentage of mastitis based on DMSCC at different levels in goats and cattle

Levels	Category	Frequency (N)	Percentage (%)
Goat			
Overall in goats (N=40)	Normal	17	42
	Subclinical mastitis	13	32
	Clinical mastitis	10	25
Right quarters (N=40)	Normal	22	55
	Subclinical mastitis	11	27
	Clinical mastitis	7	17
Left quarters (N=40)	Normal	18	45
	Subclinical mastitis	14	35
	Clinical mastitis	8	20
Cattle			
Overall in cattle (N=10)	Normal	2	20
	Subclinical mastitis	3	30
	Clinical mastitis	5	50
Fore quarters (N=20)	Normal	6	30
	Subclinical mastitis	7	35
	Clinical mastitis	7	35
Rear quarters (N=20)	Normal	4	20
	Subclinical mastitis	12	60
	Clinical mastitis	4	20
Right fore quarters (N=10)	Normal	3	30
	Subclinical mastitis	3	30
	Clinical mastitis	4	40
Right rear quarters (N=10)	Normal	2	20
	Subclinical mastitis	5	50
	Clinical mastitis	3	30
Left fore quarters (N=10)	Normal	3	30
	Subclinical mastitis	4	40
	Clinical mastitis	3	30
Left rear quarters (N=10)	Normal	2	20
	Subclinical mastitis	7	70
	Clinical mastitis	1	10

4.1 History and basic physical parameters

All goats and cattle were lactating in this study, where average ages were 4 ± 1.2 and 6 ± 2 years respectively. The average body weight in goats and cattle were 35 ± 4.3 and 400 ± 50 kg respectively. The mean value of rectal temperature of goat and cattle was 102 ± 1.5^0 F and 101 ± 0.5^0 F respectively. In goats and cattle, the mean of heart rate and respiration rate cattle were 75 ± 8.2 and 25 ± 5.5/minute, and 70 ± 5.2 and 22 ± 3.5/minute respectively. The conjunctival mucous membrane of all animals were slightly pale to pink in color.

4.2 Clinical evaluations of mammary gland

A detailed clinical examinations of udder, teats and supramammary lymph nodes were performed of all animals.

4.2.1 Clinical examination of udder

On clinical examination of udder in goats, 32.5% (13/40) and 10% (4/40) normal animal had symmetrical and asymmetrical udders respectively; 15% (6/40) and 17.5% (7/40) animals affected with subclinical mastitis had symmetrical and asymmetrical udders respectively and 7.5% (3/40) and 17.5% (7/40) animals affected with clinical mastitis had symmetrical and asymmetrical udders respectively (P <0.05) (Graph 4.1). In total of 40 goats, 24 udders were pendulous in shape and 16 udders were spherical in shape. In normal animals, 15% (6/40) and 27.5% (11/40) udders were pendulous and spherical in shape respectively, whereas in subclinical mastitis, 27.5% (11/40) and 5% (2/40) udders were pendulous and spherical-shaped. On the other hand, in clinical mastitis, 17.5% (7/40) and 7.5% (3/40) udders were pendulous and spherical in shape respectively (P <0.05) (Graph 4.2). During the evaluation of udder, visible abnormalities were observed in clinical mastitis whereas 80% (8/10) udders were swollen, 20% (2/10) were indurated, 60% (6/10) were painful on palpation, 60% (6/10) were warm and 20% (2/10) were cold on touch. Abnormalities of the skin of the udder were evident in 30% (3/10) udder whereas erythema was found in 10% and bluish discoloration was found in 20% (2/10) skin (Graph 4.3). Udder parenchyma were palpably normal in 30/40 (75%) goats and lesions in the glandular parenchyma were evident by palpation in 10/40 (25%).
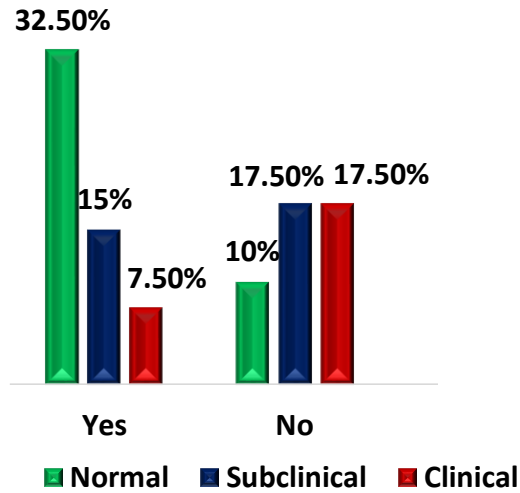
Graph 4.1: The relationship between symmetry of the udder and mastitis in goats
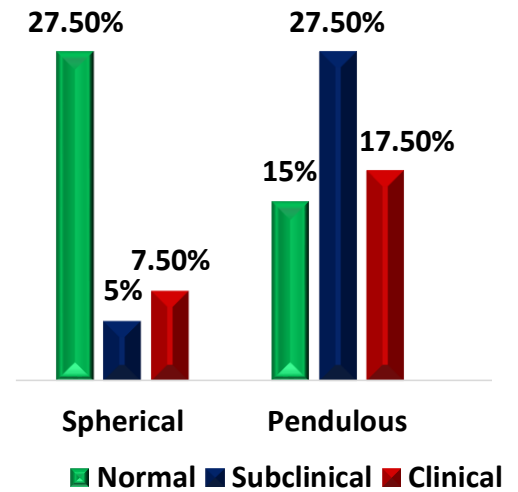
Graph 4.2: The relationship between shape of the udder and mastitis in goats
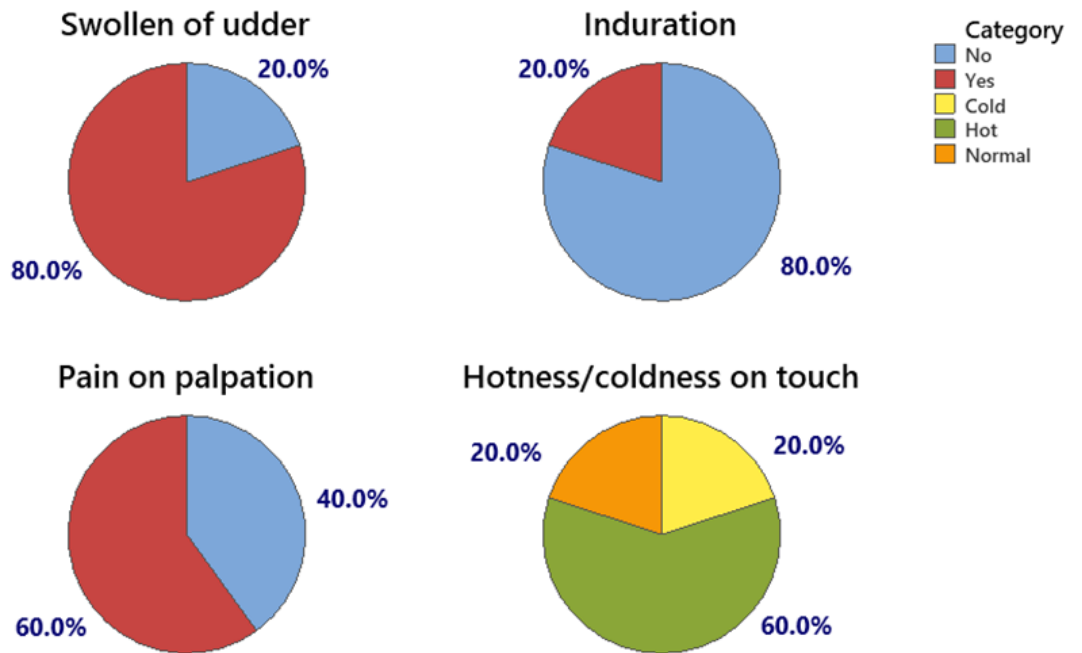
Graph 4.3: The physical abnormalities of the udder during clinical mastitis in goats

On clinical examination of udder in cattle, 20% (2/10) and 30% (3/10) normal cows and cows affected with subclinical mastitis had symmetrical udders respectively. On the other hand, symmetrical and asymmetrical udders were found 10% (1/10) and 40% (4/10) cows affected clinical mastitis respectively (Graph 4.4). In total of 10 cows, all udders were spherical in shape. During the evaluation of udder, visible abnormalities were observed in clinical mastitis whereas 80% (4/5) udders were swollen, 80% (4/5) were painful on palpation and 80% (4/5) were warm on touch (Graph 4.5). Udder parenchyma were palpably normal in 50% (5/10) cattle and lesions in the glandular parenchyma were evident by palpation in 50% (5/10) cattle.
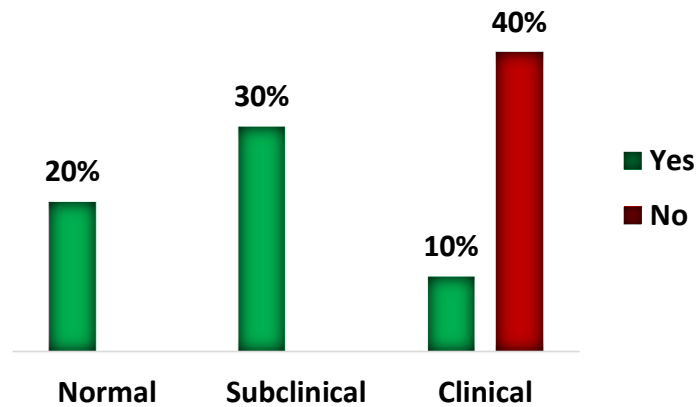
Graph 4.4: The relationship between symmetry of the udder and mastitis in cattle
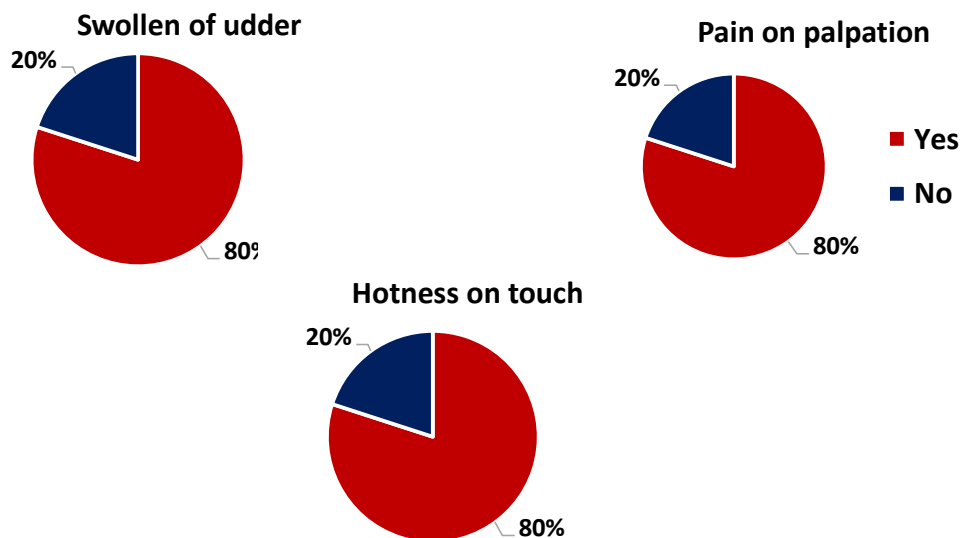
Graph 4.5: The physical abnormalities of the udder during clinical mastitis in cattle

4.2.2 Clinical examination of teat

On clinical examination of teats in goats, 30% (12/40) and 12.5% (5/40) normal animal had symmetrical and asymmetrical teats respectively; 17.5% (7/40) and 15% (6/40) animals affected with subclinical mastitis had symmetrical and asymmetrical teats respectively and 2.5% (1/40) and 22.5% (9/40) animals affected with clinical mastitis had symmetrical and asymmetrical teats respectively (P <0.05) (Graph 4.6). In total, 17 teats were bottle in shape, 13 teats were funnel in shape and 10 teats were cylindrical in shape. In normal animals, 7.5% (3/40), 25% (10/40) and 10% (4/40) teats were bottle, funnel and cylindrical in shape respectively whereas 17.5% (7/40), 5% (2/40) and 10% (4/40) teats were bottle, funnel and cylindrical in shape animals affected with subclinical mastitis respectively, and 17.5% (7/40), 2.5% (1/40) and 5% (2/40) teats were bottle, funnel and cylindrical in shape animals affected with clinical mastitis respectively (P <0.05) (Graph 4.7). During the evaluation of teat, visible abnormalities were observed in clinical mastitis whereas swollen of teats were found in 80% (8/10) goats, 60% (6/10) goats were found painful teats on palpation, 60% (6/10) were warm and 20% (2/10) were cold on touch. Abnormalities of the skin of teats were evident in 30% (3/10) goats whereas erythema was found in 10% (1/10) and bluish discoloration found in 20% (2/10) goats. Teats were palpably normal in 30/40 (75%) goats and lesions in teats were evident by palpation in 10/40 (25%) whereas milk flow obstructions were found in 3/10 (7.5%) goats.
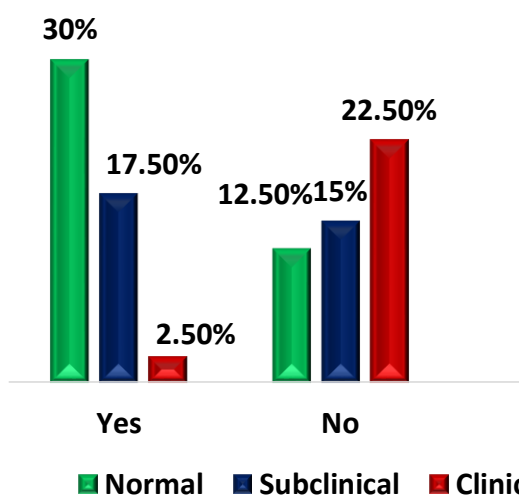
Graph 4.6: The relationship between symmetry of teat and mastitis in goats
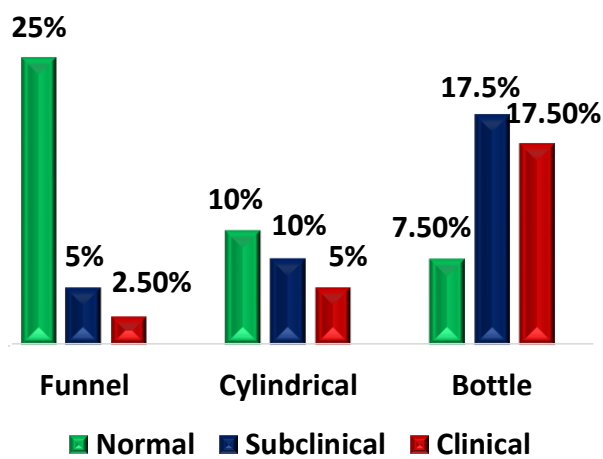
Graph 4.7: The relationship between shape of teat and mastitis in goats

34

Figure 4.1: Changes in the color of skin in the udder and teat during clinical mastitis of goats

On clinical examination of teats in cattle, 20% (2/10) normal cows and 30% (3/10) cows affected with subclinical mastitis had symmetrical teats. On the other hand, symmetrical and asymmetrical teats were found 20% (2/10) and 30% (3/10) cows affected with clinical mastitis respectively (Graph 4.8). In total of 10 cows, all teats were cylindrical in shape. During the evaluation of teat, visible abnormalities were observed in clinical mastitis whereas swollen of teats were found in 60% (3/5) cattle, 60% (6/10) cattle were found painful teats on palpation, 80% (6/10) cattle had warm on touch. Teats were palpably normal in 70% (7/10) cattle and lesions in the teats were evident by palpation in 30% (3/10) cattle whereas milk flow obstructions were found in 3/10 (30%) cattle.
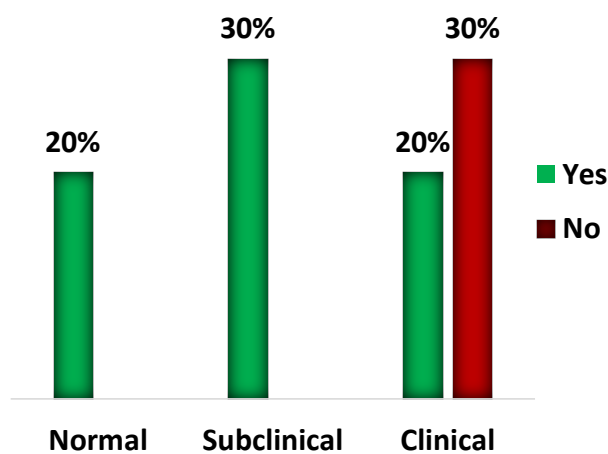
Graph 4.8: The relationship between symmetry of teat and mastitis in cattle
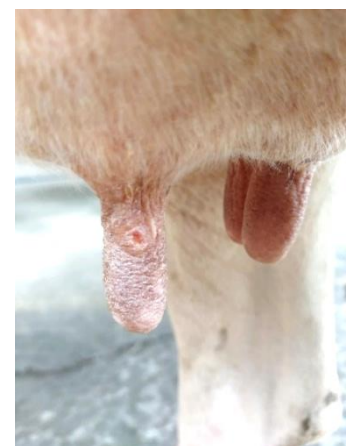
Figure 4.2: Asymmetry of teat during clinical mastitis in cattle

4.2.3 Clinical examination of supramamary lymph nodes

The supramammary lymph nodes in all goats were clinically examined through palpation whereas the lymph nodes of 22.5% (9/40) goats were markedly enlarged which were affected with clinical mastitis. Similarly, the supramammary lymph nodes in all cattle were clinically examined through palpation whereas the lymph nodes of 50% (5/10) cattle were markedly enlarged affected with clinical mastitis (Graph 4.9).
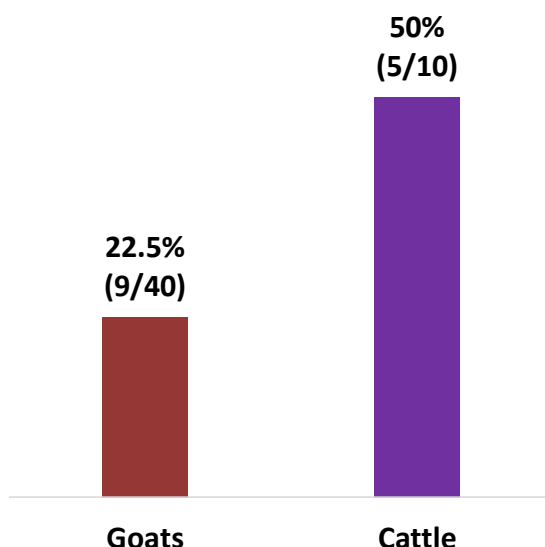
Graph 4.9: The percentages of enlargement of supramammary lymph nodes during clinical mastitis in goats and cattle

Figure 4.3: Enlargement of supramammary lymph nodes during clinical mastitis in goats

4.2.3 Clinical examination of milk

No milk clots or abnormalities were evident in 30/40 (75%) goats. Milk abnormalities were evident in 10 goats, which were affected with clinical mastitis. Among the abnormalities, 40% (4/10) were white clotted, 30% (3/10) were bloody milk, 30% (3/10) were watery milk (Graph 4.10).

On the other hand, no milk clots or abnormalities were evident in 50% (5/10) cattle. Milk abnormalities were evident in 5 cattle, which were affected with clinical mastitis. Among the abnormalities, 60% (3/5) were white clotted and 40% (2/5) were watery milk (Graph 4.11).
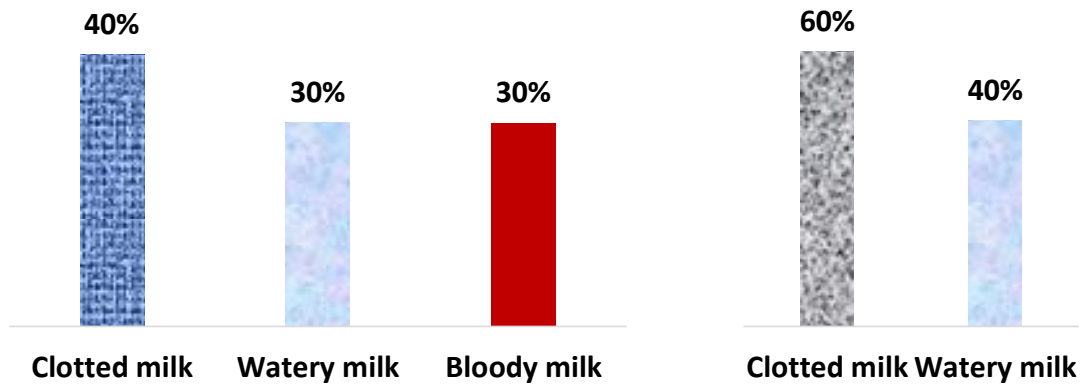
Graph 4.10: Physical abnormalities of milk were found during clinical mastitis in goats

Graph 4.11: Physical abnormalities of milk were found during clinical mastitis in cattle
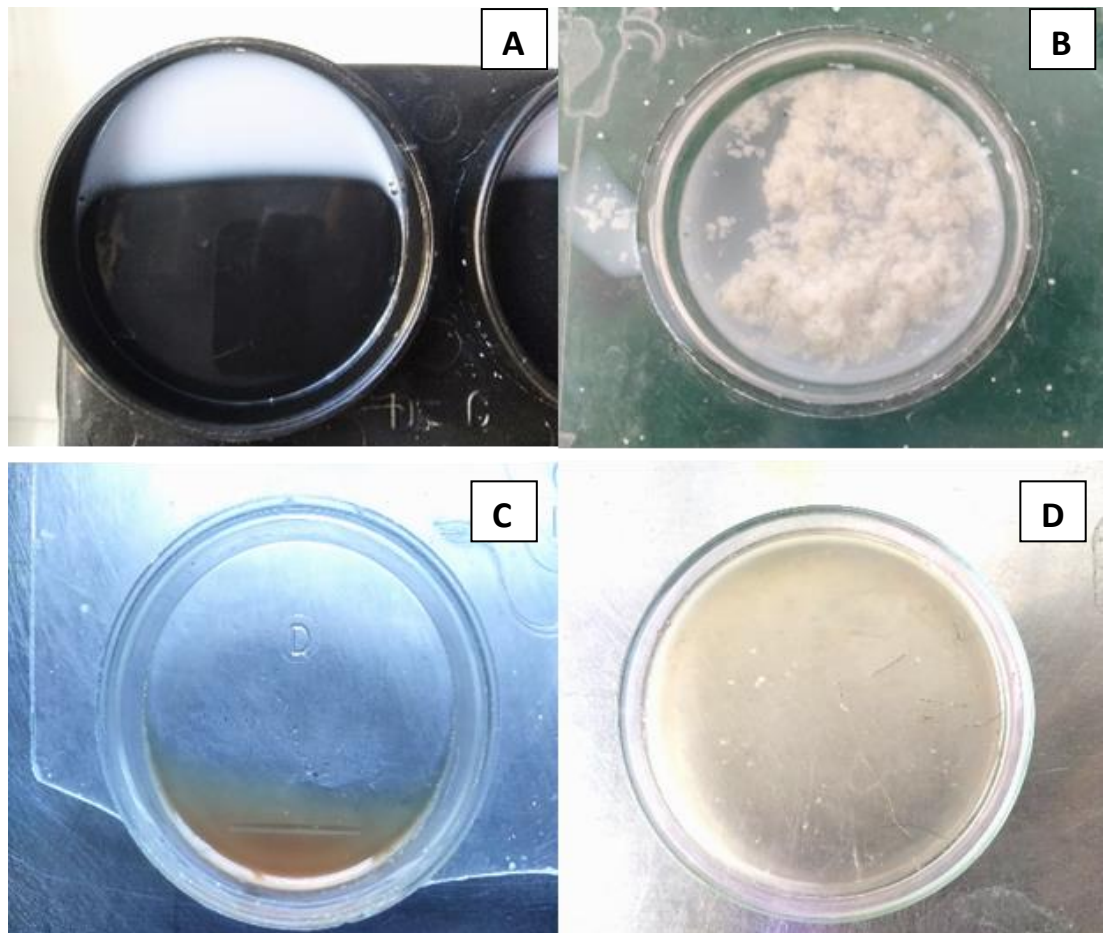
Figure 4.4: The physical findings of milk: A) normal, B) clotted, C) bloody, D) watery milk just after collection.

4.3 Ultrasonographical examination of the mammary gland

4.3.1 The ultrasonographical measurements of teat parameters in goats

The ultrasonographical measurements of teat parameters of normal, subclinical and clinical mastitis in goats are presented in Table 4.2.

In goats, the mean values of teat canal length of right quarter in normal teat, affected with subclinical mastitis and clinical mastitis in goats were 5.4 ± 0.8 mm, 6.6 ± 0.9 mm and 7.7 ± 1.3 mm respectively. The mean values of teat canal length of left quarter in normal teat, affected with subclinical and clinical mastitis in goats were 5.2 ± 0.7 mm, 5.9 ± 1.0 mm and 6.9 ± 1.1 mm respectively. The mean values of teat canal length were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of teat canal length in normal teat was significantly ($p<0.05$) shorter than the both teats affected with subclinical and clinical mastitis in goats. However, there was no significant differences between subclinical and clinical mastitis in goats.

The mean values of teat canal diameter of right quarter in normal teat, affected with subclinical mastitis and clinical mastitis in goats were 1.6 ± 0.2 mm, 1.9 ± 0.3 mm and 2.1 ± 0.4 mm respectively. The mean values of teat canal diameter of left quarter in normal teat, affected with subclinical and clinical mastitis were 1.6 ± 0.4 mm, 1.8 ± 0.3 mm and 2.2 ± 0.3 mm respectively. The mean values of teat canal diameter were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of teat canal diameter in goats affected with clinical mastitis was significantly ($p<0.05$) wider than both the normal teat and teat affected with subclinical mastitis in goats. Similarly, the mean value of teat canal diameter was significantly ($p<0.05$) wider in teat affected with subclinical mastitis than normal goats.

The mean values of teat wall thickness of normal teat, affected with subclinical mastitis and clinical mastitis in right quarter of goats were 4.2 ± 0.8 mm, 5.0 ± 0.8 mm and 6.3 ± 1.0 mm respectively. Similarly, the mean values of teat wall thickness of normal teat, affected with subclinical and clinical mastitis in left quarter of goats were 4.1 ± 0.8 mm, 4.9 ± 0.7 mm and 6.0 ± 0.9 mm respectively. The mean values of

teat wall thickness were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of teat wall thickness in goats affected with clinical mastitis was significantly ($p<0.05$) increased than both the normal teat and affected with subclinical mastitis in goats. Similarly, the mean value of teat wall thickness was also significantly increased in teat affected with subclinical mastitis than normal goats.

The mean values of teat cistern diameter of normal teat, affected with subclinical mastitis and clinical mastitis in right quarter of goats were 18.8 ± 3.9 mm, 18.9 ± 4.3 mm and 16.5 ± 4.7 mm respectively. Similarly, the mean values of teat cistern diameter of normal teat, affected with subclinical and clinical mastitis in left quarter of goats were 18.7 ± 3.7 mm, 17.5 ± 5.9 mm and 16.0 ± 3.2 mm respectively. The mean values of teat cistern diameters were not significantly ($p<0.05$) different among normal, subclinical and clinical mastitis in goats.

Table 4.2: The ultrasonographical measurement of teat parameters in goats

Traits	Right Quarter			Left Quarter			*P-*value
	Normal, N=22 (mean ± SD)	Subclinical, N=11 (mean ± SD)	Clinical, N=7 (mean ± SD)	Normal, N=18 (mean ± SD)	Subclinical, N=14 (mean ± SD))	Clinical, N=8 (mean ± SD))	
TCL (mm)	5.4 ± 0.8^{c}	6.6 ± 0.9^{ab}	7.7 ± 1.3^{a}	5.2 ± 0.7^{c}	5.9 ± 1.0^{ab}	6.9 ± 1.1^{a}	0.001
TCD (mm)	1.6 ± 0.2^{c}	1.9 ± 0.3^{b}	2.1 ± 0.4^{a}	1.6 ± 0.4^{c}	1.8 ± 0.3^{b}	2.2 ± 0.3^{a}	0.001
TWT (mm)	4.2 ± 0.8^{c}	5.0 ± 0.8^{b}	6.3 ± 1.0^{a}	4.1 ± 0.8^{c}	4.9 ± 0.7^{b}	6.0 ± 0.9^{a}	0.001
TcD (mm)	18.8 ± 3.9	18.9 ± 4.3	16.5 ± 4.7	18.7 ± 3.7	17.5 ± 5.9	16.0 ± 0.9	NA

TCL= Teat canal length, TCD= Teat canal diameter, TWT= Teat wall thickness, TcD= Teat cistern diameter. Different superscript letters a, b, c indicate statistically significant (p<0.05), NA indicates non-significant

Table 4.3: The ultrasonographical measurements of teat parameters in cattle

The ultrasonographic measurement of teat parameters among normal, subclinical, and clinical mastitis in cattle are presented in Table 4.3.

In cattle, the mean values of teat canal length of right fore quarter in normal teat, affected with subclinical mastitis and clinical mastitis were 7.2 ± 1.2 mm, 9.1 ± 1.1 mm and 9.6 ± 2.1 mm respectively. The mean values of teat canal length of right hind quarter in normal teat, affected with subclinical and clinical mastitis were 8.2 ± 1.1 mm, 9.4 ± 1.0 mm and 9.7 ± 1.9 mm respectively. The mean values of teat canal length of left fore quarter in normal teat, teat affected with subclinical and clinical mastitis were 7.9 ± 0.6 mm, 8.9 ± 1.6 mm and 9.3 ± 1.7 mm respectively. Similarly, the mean values of teat canal length of left hind quarter in normal teat, affected with subclinical and clinical mastitis were 8.3 ± 1.3 mm, 9.9 ± 1.7 mm and 10.9 ± 0.0 mm respectively. However, the mean values of teat canal length were not significantly ($p<0.05$) different among normal, subclinical and clinical mastitis in cattle.

The mean values of teat canal diameter of right fore quarter in normal teat, affected with subclinical mastitis and clinical mastitis in goats were 2.3 ± 0.3 mm, 2.5 ± 0.7 mm and 3.2 ± 0.5 mm respectively. The mean values of teat canal diameter of right hind quarter in normal teat, teat affected with subclinical and clinical mastitis were 2.5 ± 0.6 mm, 2.7 ± 0.7 mm and 2.9 ± 0.4 mm respectively. The mean values of teat canal diameter of left fore quarter in normal teat, affected with subclinical and clinical mastitis were 2.6 ± 0.1 mm, 2.7 ± 0.4 mm and 3.2 ± 0.3 mm respectively. The mean values of teat canal diameter of left hind quarter in normal teat, affected with subclinical and clinical mastitis were 2.7 ± 0.3 mm, 2.9 ± 0.3 mm and 2.9 ± 0.0 mm respectively. However, the mean values of teat canal diameter were not significantly ($p<0.05$) different among normal, subclinical and clinical mastitis in cattle.

The mean values of teat wall thickness of normal teat, affected with subclinical mastitis and clinical mastitis in right fore quarter were 7.3 ± 0.7 mm, 7.8 ± 0.2 mm and 8.5 ± 1.5 mm respectively. The mean values of teat wall thickness of normal teat, affected with subclinical and clinical mastitis in right hind quarter were 7.2 ± 1.2 mm, 7.9 ± 1.1 mm and 8.4 ± 1.7 mm respectively.

The mean values of teat wall thickness of normal teat, affected with subclinical and clinical mastitis in left fore quarter were 7.3 ± 0.6 mm, 7.6 ± 2.3 mm and 9.5 ± 1.7 mm respectively. The mean values of teat wall thickness of normal teat, affected with subclinical and clinical mastitis in left hind quarter were 6.2 ± 0.6 mm, 7.0 ± 1.6 mm and 8.4 ± 0.0 mm respectively. However, the mean values of teat wall thickness were not significantly ($p<0.05$) different among normal, subclinical and clinical mastitis in cattle.

The mean values of teat cistern diameter of normal teat, affected with subclinical mastitis and clinical mastitis in right fore quarter were 9.4 ± 1.7 mm, 8.7 ± 0.6 mm and 8.2 ± 2.3 mm respectively. The mean values of teat cistern diameter of normal teat, teat affected with subclinical and clinical mastitis in right hind quarter were 10.1 ± 1.1 mm, 8.9 ± 3.7 mm and 9.7 ± 3.2 mm respectively. The mean values of teat cistern diameter of normal teat, affected with subclinical and clinical mastitis in left fore quarter were 9.0 ± 1.9 mm, 8.5 ± 1.7 mm and 8.6 ± 1.0 mm respectively. The mean values of teat cistern diameter of normal teat, affected with subclinical and clinical mastitis in left hind quarter were 10.8 ± 0.4 mm, 8.2 ± 1.9 mm and 8.8 ± 0.0 mm respectively. However, the mean values of teat cistern diameters were not significantly ($p>0.05$) different among normal, subclinical and clinical mastitis in cattle.

Table 4.3: The ultrasonographical measurement of teat parameters in cattle

Traits	Right Fore Quarter			Right Rear Quarter			Left Fore Quarter			Left Rear Quarter			P-Value
	Normal, N= 3 (mean ± SD)	Subclinical, N= 3 (mean ± SD)	Clinical, N= 4 (mean ± SD)	Normal, N= 2 (mean ± SD)	Subclinical, N= 5 (mean ± SD)	Clinical, N= 3 (mean ± SD)	Normal, N= 3 (mean ± SD)	Subclinical, N=4 (mean ± SD)	Clinical, N= 3 (mean ± SD)	Normal, N= 2 (mean ± SD)	Subclinical, N= 7 (mean ± SD)	Clinical, N= 1 (mean ± SD)	
TCL (mm)	7.2 ± 1.2	9.1 ± 1.1	9.6 ± 2.1	8.2 ± 1.1	9.4 ± 1.0	9.7 ± 1.9	7.9 ± 0.6	8.9 ± 1.6	9.3 ± 1.7	8.3 ± 1.3	9.9 ± 1.7	10.9 ± 0.0	NA
TCD (mm)	2.3 ± 0.3	2.5 ± 0.7	3.2 ± 0.5	2.5 ± 0.6	2.7 ± 0.7	2.9 ± 0.4	2.6 ± 0.1	2.7 ± 0.4	3.2 ± 0.3	2.7 ± 0.3	2.9 ± 0.3	2.9 ± 0.0	NA
TWT (mm)	7.3 ± 0.7	7.8 ± 0.2	8.5 ± 1.5	7.2 ± 1.2	7.9 ± 1.1	8.4 ± 1.7	7.3 ± 0.6	7.6 ± 2.3	9.5 ± 1.7	6.2 ± 0.6	7.0 ± 1.6	8.4 ± 0.0	NA
TcD (mm)	9.4 ± 1.7	8.7 ± 0.6	8.2 ± 2.3	10.1 ± 1.1	8.9 ± 3.7	9.7 ± 3.2	9.0 ± 1.9	8.5 ± 1.7	8.6 ± 1.0	10.8 ± 0.4	8.2 ± 1.9	8.8 ± 0.0	NA

TCL= Teat canal length, TCD= Teat canal diameter, TWT= Teat wall thickness, TcD= Teat cistern diameter. NA indicates statistically non-significant.

4.3.3 The ultrasonographical examination of udder parenchyma in goats

The echotextures of mammary parenchymal tissues of right quarter were homogenous in 55% (22/40) normal goats, homogenous and heterogeneous presenting in 22.5% (9/40) and 5% (2/40) subclinical mastitis respectively, and heterogeneous in 17.5% (7/40) clinical mastitis in goats (Graph 4.12A). Although, there was not found heterogeneous and hyperechogenecity in normal glandular tissues, the heterogeneous echotexture along with partly hyperechoic and partly hypoechoic echogenicity were found in clinical mastitis in goats. However, the echotextures of mammary parenchymal tissues were significantly different ($P<0.01$) among normal, subclinical and clinical mastitis in goats. The hyperechoic spots in mammary tissues were absent in 55% (22/40) normal quarter, hyperechoic spots were present and absent in 5% (2/40) and 22.5% (9/40) subclinical mastitis respectively, and present in (17.5%, 7/40) all cases of clinical mastitis in goats (Graph 4.12B). The hyperechoic spots in mammary tissues were significantly different ($P<0.01$) among normal, subclinical and clinical mastitis in goats.
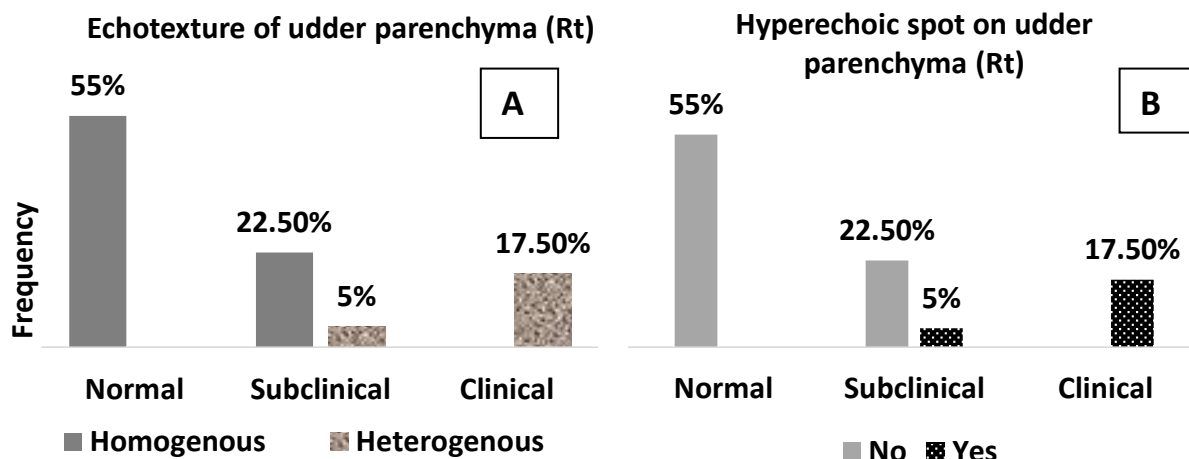
Graph 4.12: A) The findings of echotexture, B) hyperechoic spots on udder parenchyma in goats (right quarter)

The echotextures of mammary parenchymal tissues of left quarter were homogenous in 45% (18/40) normal goats, homogenous and heterogeneous showing on 30% (12/40) and 5% (2/40) subclinical mastitis respectively, and heterogeneous in 20% (8/40) clinical mastitis in goats (Graph 4.13A). Although, there was not found heterogeneous and hyperechogenecity in normal glandular tissues, the heterogeneous echotexture along with partly hyperechoic and partly hypoechoic echogenicity were found in clinical mastitis of goats. However, the echotextures of mammary parenchymal tissues were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in goats. The hyperechoic spots in mammary tissues were absent in 45% (18/40) normal quarter, hyperechoic spots were present and absent in 5% (2/40) and 30% (8/40) subclinical mastitis respectively, and present in 20% (8/40) clinical mastitis in goats (Graph 4.13B). The hyperechoic spots in mammary tissues were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in goats.
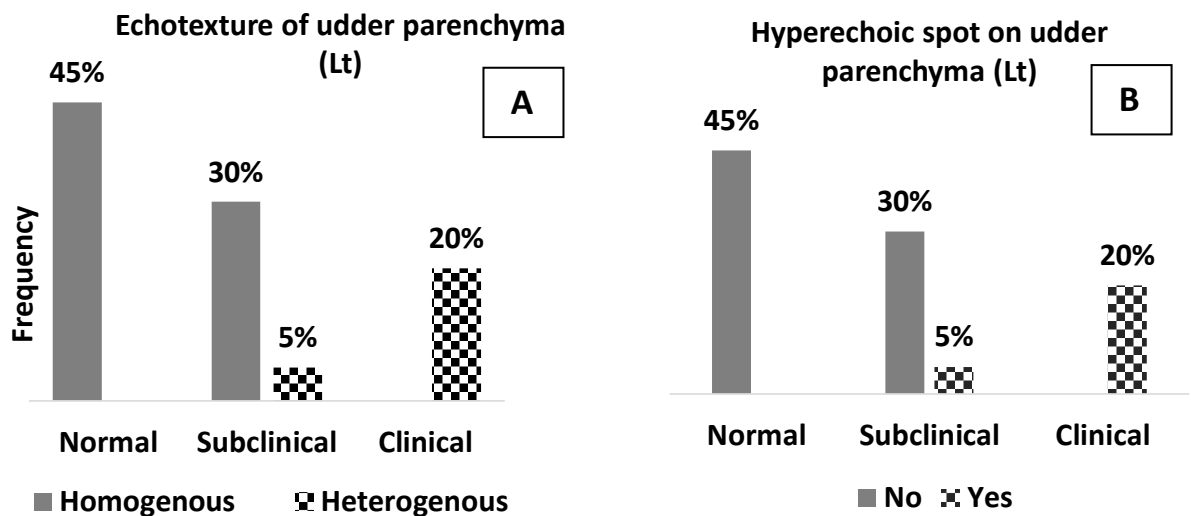
Graph 4.13: A) The findings of echotexture and B) hyperechoic spots on udder parenchyma in goats (left quarter)
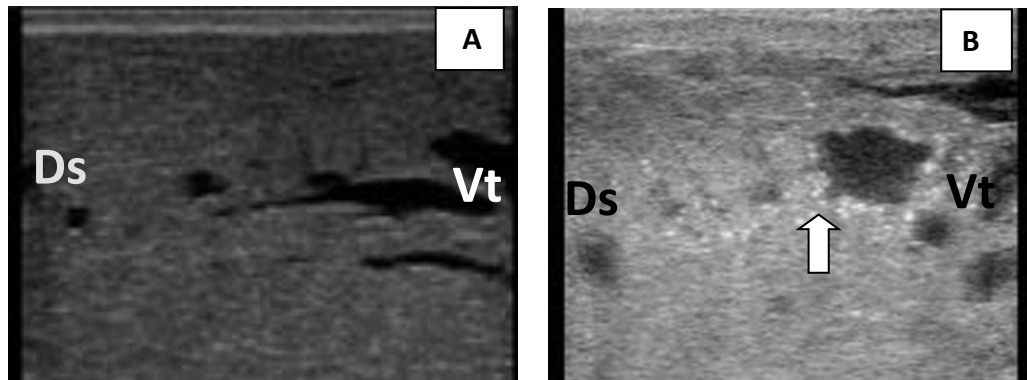
Figure 4.5: A) Normal udder parenchyma and B) heterogenous and hyperechoic spots on udder parenchyma during mastitis in goats (Ds=Dorsal, Vt=Ventral)

Although, the lactiferous ducts in udder parenchymal tissues of right quarter were clearly visible in 55% (22/40) normal goats, and 27.5% (11/40) goats affected with subclinical mastitis, the lactiferous ducts were hardly visible in 17.5% (7/40) goats affected with clinical mastitis (Graph 4.14A). On the other hand, the lactiferous ducts in udder parenchyma tissues of left quarter were clearly visible in 45% (18/40) normal goats and 35% (14/40) goats affected with subclinical mastitis, the lactiferous ducts were hardly visible in 20% (8/40) goats affected with clinical mastitis (Graph 4.14B). The scanning of lactiferous ducts was significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in goats.
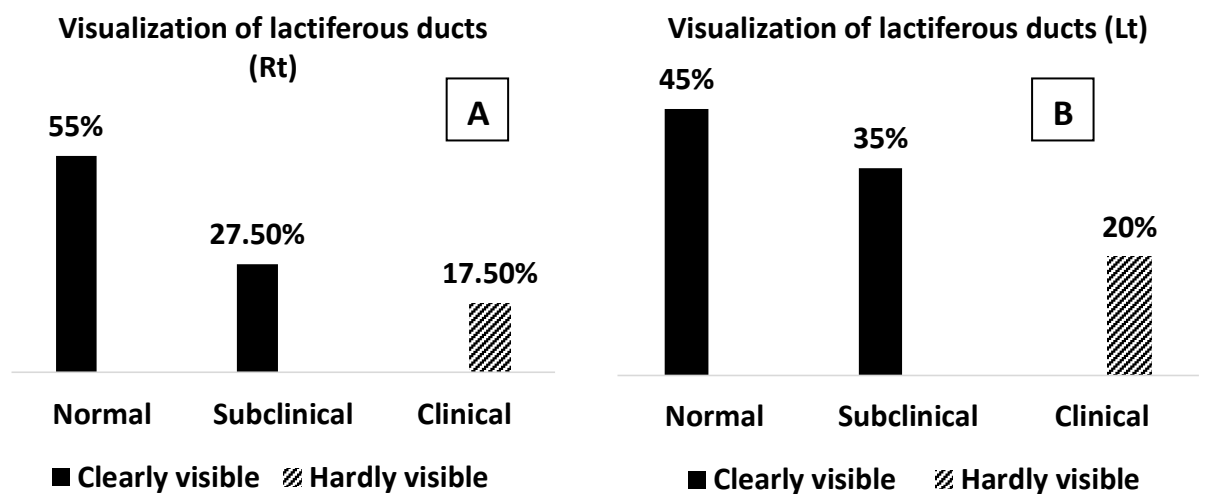
Graph 4.14: The ultrasonographic findings of lactiferous ducts in goats: A) right quarter and B) left quarter
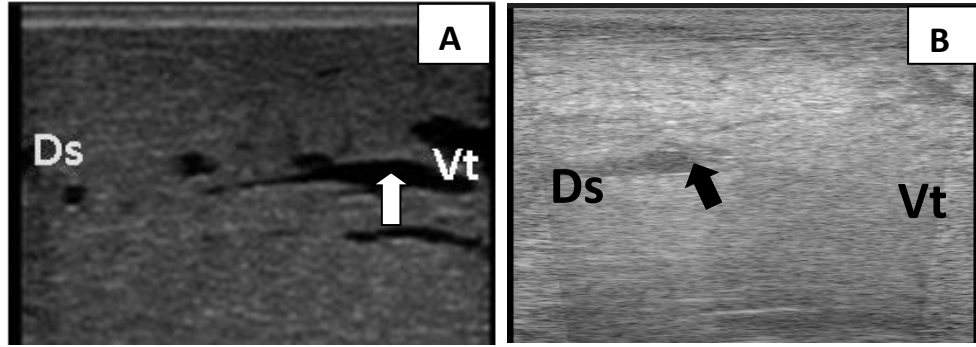
Figure 4.6: The visualization of lactiferous ducts in goats: A) clearly visible, B) hardly visible

4.3.3 The ultrasonographical examination of udder parenchyma in cattle

In total of 40 quarters in cattle, echotextures of mammary parenchymal tissues were scanned ultrasonographically. The echotextures of mammary parenchymal tissues were homogenous in 25% (10/40) normal quarter, homogenous and heterogeneous in 40% (16/40) and 7.5% (3/40) subclinical mastitis respectively, and heterogeneous in 27.5% (11/40) clinical mastitis in cattle (Graph 4.15A). Although, there was not found heterogeneous and hyperechogenecity in normal glandular tissues, the heterogeneous echotexture along with partly hyperechoic and partly hypoechoic echogenicity were found in clinical mastitis in cattle. However, the echotextures of mammary parenchymal tissues were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in cattle. The hyperechoic spots in mammary tissues were absent in 25% (10/40) normal quarter, present and absent in 40% (16/40) and 7.5% (3/40) subclinical mastitis respectively, and present in 27.5% (11/40) quarter affected clinical mastitis in cattle (Graph 4.15B). The hyperechoic spots in mammary tissues were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in cattle.
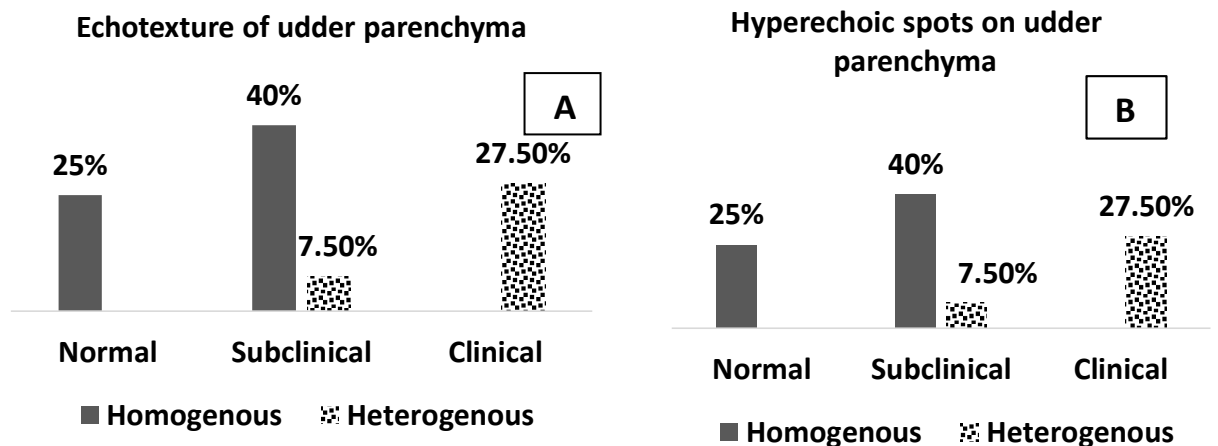
Graph 4.15: A) The findings of echotexture, B) hyperechoic spots on udder parenchyma in cattle
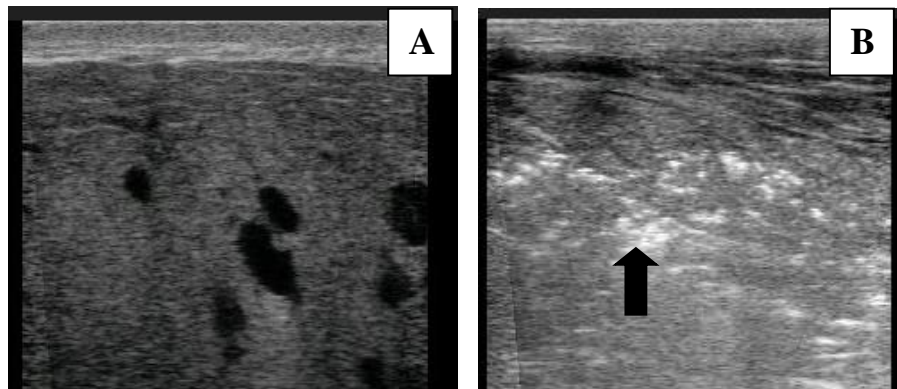
Figure 4.7: A) Normal udder parenchyma, B) heterogenous and hyperechoic spots on udder parenchyma during mastitis in cattle.

Although, the lactiferous ducts in udder parenchymal tissues were clearly visible in 25% (10/40) and 47.5% (19/40) normal quarters and quarters affected with subclinical mastitis respectively, but the lactiferous ducts were hardly visible in (27.5%, 11/40) all quarters affected with clinical mastitis in cattle (Graph 4.16). The scanning of lactiferous ducts in parenchymal tissues was significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in cattle.
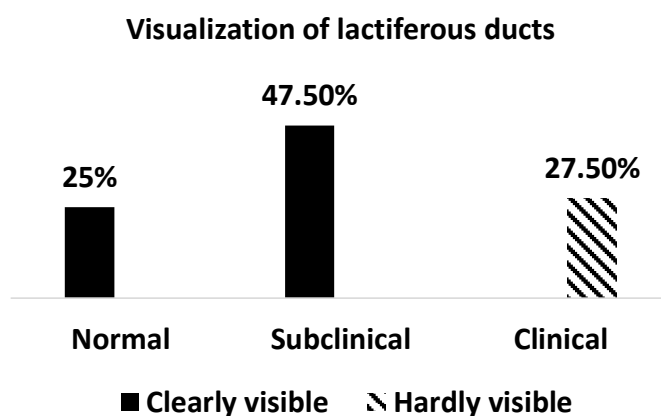
Graph 4.16: The ultrasonographic findings of lactiferous ducts in cattle
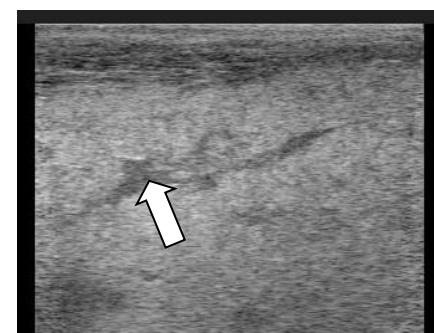
Figure 4.8: The lactiferous ducts are hardly visible during clinical mastitis in cattle

4.3.4 The ultrasonographical examination of gland cistern in goats

The contents of gland cisterns of right quarter were found anechoic echogenicity in 55% (22/40) normal quarters and 27.5% (11/40) quarters affected with subclinical mastitis, although hypoechoic echogenicity was found in 17.5% (7/40) quarters affected with clinical mastitis (Graph 4.17A). The contents of gland cisterns were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in goats.

On the other hand, the contents of gland cisterns of left quarter were found anechoic echogenicity in 45% (18/40) normal quarters and 35% (14/40) quarters affected with subclinical mastitis, although hypoechoic echogenicity was found in 20% (8/40) goats affected with clinical mastitis (Graph 4.17B). The contents of gland cisterns were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in goats.
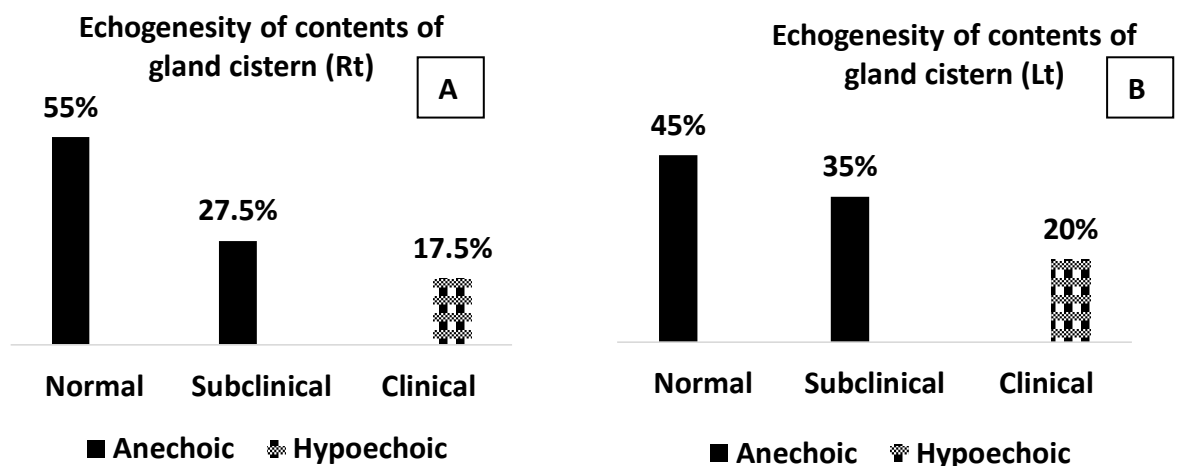
Graph 4.17: The findings of gland cistern in goats: A) right B) left quarter
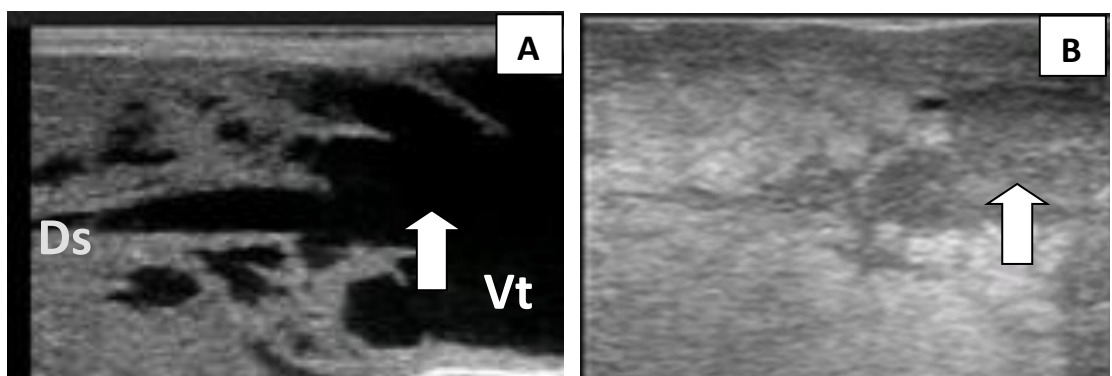
Figure 4.9: The findings of gland cistern in goats: A) Anechoic contents in gland cistern (normal), B) Hypo to hyperechoic flakes mixed with anechoic fluids in gland cistern during clinical mastitis

4.3.5 The ultrasonographical examination of gland cistern in cattle

In total of 40 quarters in cattle, contents of gland cisterns were scanned ultrasonographically. The contents of gland cisterns were found anechoic echogenicity in 25% (10/40) normal quarters and 47.5% (19/40) quarters affected with subclinical mastitis although hypoechoic echogenicity was found in 27.5% (11/40) quarter affected with clinical mastitis in cattle (Graph 4.18). The contents of gland cisterns were significantly different ($p<0.01$) among normal, subclinical and clinical mastitis in cattle.
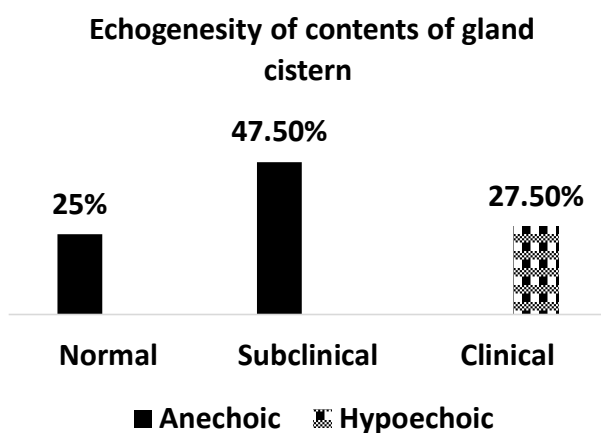
Graph 4.18: The ultrasonographic findings of the gland cistern in cattle.
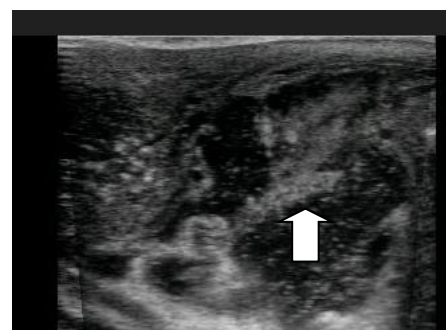
Figure 4.10: The hypo to hyperechoic flakes mixed with anechoic fluids in gland cistern during clinical mastitis

4.3.6. The ultrasonographical examination of teat wall in goats

Well visualization of the teat wall along with clear imaging of a triple-layered structure was attained. The contour of teat wall lining of right teat was regular in 45% (18/40) normal goats and 15% (6/40) goats affected with subclinical mastitis, whereas irregular teat wall lining was found in 10% (4/10) normal goats, 12.5% (5/40) goats affected with subclinical mastitis and 17.5% (7/40) goats affected with clinical mastitis (Graph 4.19A). Besides, the contour of teat wall lining of left teat was regular in 37.5% (15/40) normal teats, 25% (10/40) and 5% (2/40) teats affected with subclinical and clinical mastitis respectively whereas irregular teat wall lining was found in 7.5% (3/40) normal teats, 10% (4/40) teats affected with subclinical mastitis and 15% (6/40) teats affected with clinical mastitis (Graph 4.19B). The contour of teat

wall lining was significantly different ($p<0.05$) among normal, subclinical and clinical mastitis in goats.

Contour of teat wall lining (Rt)

A

45%
10%
15% 12.50%
17.50%

Normal   Subclinical   Clinical

⫾ Regular   ≋ Irregular

Contour of teat wall lining (Lt)

B

37.50%
25%
15%
7.50%
5%
15%

Normal   Subclinical   Clinical
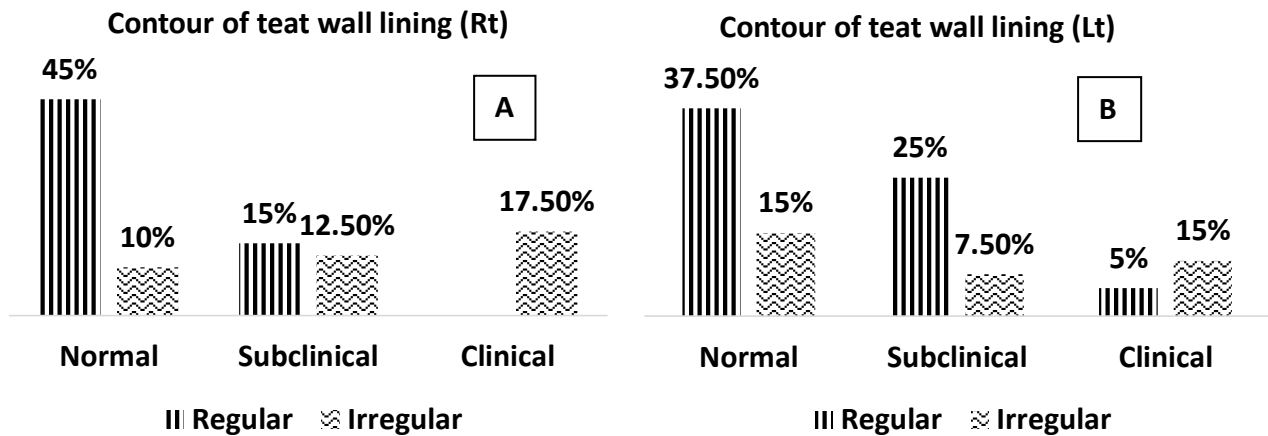
⫾ Regular   ≋ Irregular

Graph 4.19: The ultrasonographic findings of regular and irregular lining of teat wall in goats: A) right, B) left quarter
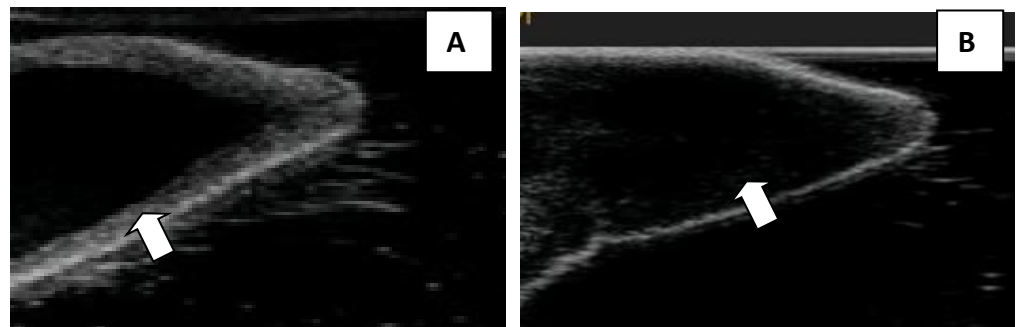
Figure 4.11: Ultrasound images of teat wall lining in goats: A) Regular lining in normal goats, B) Irregular lining in subclinical mastitis

4.3.7 The ultrasonographical examination of teat wall in cattle

In total of 40 teats in cattle, the contour of teat wall lining was regular in 17.5% (7/40) normal teats and 25% (10/40) teats affected with subclinical mastitis in cattle. On the other hand, irregular teat wall lining was found in 7.5% (3/40) normal teats, 22.5% (9/40) teats affected with subclinical mastitis and 27.5% (11/40) teats affected with clinical mastitis (Graph 4.20). The contour of teat wall lining was significantly different ($p<0.05$) among normal, subclinical and clinical mastitis in goats.

Contour of teat wall lining
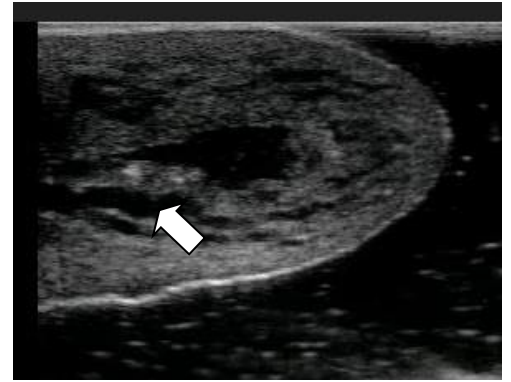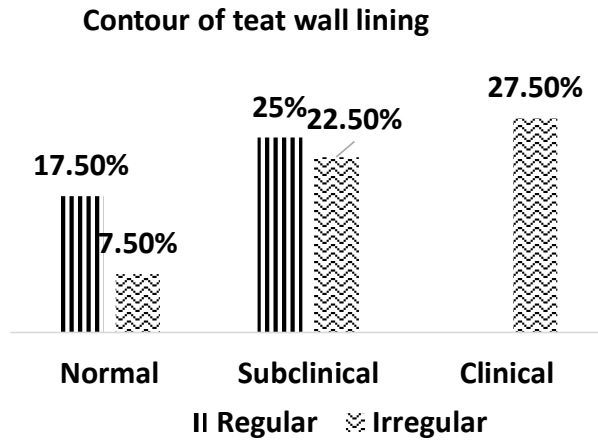
Graph 4.20: The ultrasonographic findings of contour of teat wall lining in cattle

Figure 4.12: Irregular lining of teat wall during clinical mastitis in goats.

4.3.8 The ultrasonographical examination of teat cistern in goats

The contents of teat cistern in scanning were found anechogenic in all cases of normal teats and teats affected with subclinical mastitis although hypoechogenic and/or hyperechogenic contents were found in all cases of clinical mastitis in both the right and left teat cisterns. Besides, the teat canal obstruction in scanning was found 2.5% (1/40) and 5% (2/40) in right and left teats of goats respectively (Graph 4.21).
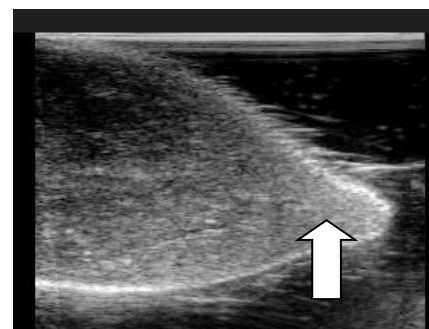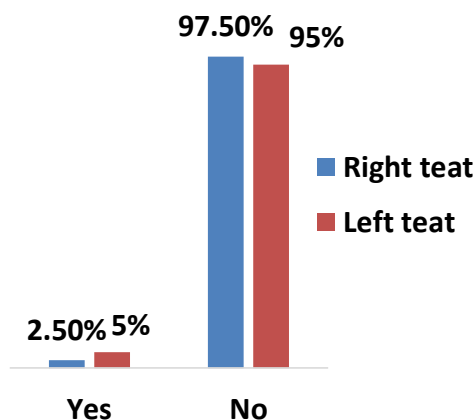
Graph 4.21: The frequency of teat canal obstruction in goats

Figure 4.13: Obstruction of teat canal in goats

4.3.9 The ultrasonographical examination of teat cistern in cattle

In cattle, the contents of teat cistern in scanning were found anechogenic in all cases of normal teats (25%, 10/40), and teats affected with subclinical mastitis (47.5%, 19/40) although hypoechogenic and/or hyperechogenic contents were found in all cases of clinical mastitis (27.5%, 11/40) in cattle. Besides, the teat canal obstruction in scanning was found in 12.5% (5/40) teats affected clinical mastitis in cattle (Graph 4.22).
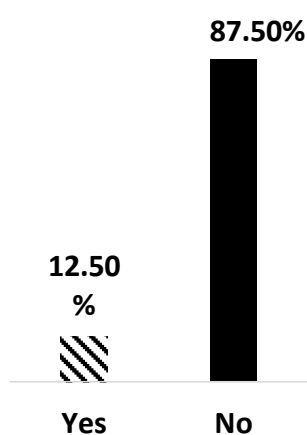
Figure 4.22: The frequency of the teat canal obstruction in cattle
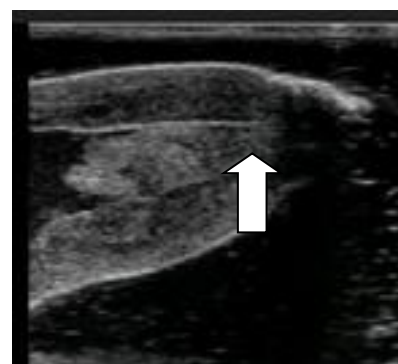
Figure 4.14: Obstruction of a teat canal in cattle

4.3.10 The ultrasonographical measurement of supramammary lymph nodes in goats

Ultrasonographical measurement of supramammary lymph nodes among normal, subclinical and clinical mastitis in goats are presented in Table 4.4.

In goats, the mean values of length and width of right supramammary lymphnodes in normal, subclinical and clinical mastitis were 15.0 ± 1.9 and 5.9 ± 0.9 mm, 17.1 ± 2.1 and 7.0 ± 1.2 mm, and 21.6 ± 2.2 and 9.6 ± 1.3 mm respectively. The mean values of length and width of left supramammary lymphnodes in normal, subclinical and clinical mastitis in goats were 14.7 ± 1.7 and 5.3 ± 0.9 mm, 16.5 ± 2.0 and 6.2 ± 1.1 mm, and 20.5 ± 2.6 and 9.1 ± 1.2 mm respectively. The mean values of length and width of right supramammary lymphnodes were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of length and width of supramammary lymphnodes were significantly

(p<0.05) increased in clinical mastitis than both the subclinical mastitis and normal goats and also increased in subclinical mastitis than the normal goats.

4.3.11 The ultrasonographical measurement of supramammary lymph nodes in cattle

Ultrasonographical measurement of supramammary lymph nodes among normal, subclinical and clinical mastitis in goats are presented in Table 4.4.

In cattle, the mean values of length and width of right supramammary lymphnodes in normal, subclinical and clinical mastitis were 52.5 ± 0.2 and 21.1 ± 1.2 mm, 75.14 ± 4.2 and 26.9 ± 1.1 mm, and 110.5 ± 6.1 and 29.8 ± 5.5 mm respectively. The mean values of length and width supramammary lymphnodes were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of length and width of supramammary lymphnodes were significantly ($p<0.05$) increased in clinical mastitis than both the subclinical mastitis and normal goats and also increased in subclinical mastitis than the normal goats.
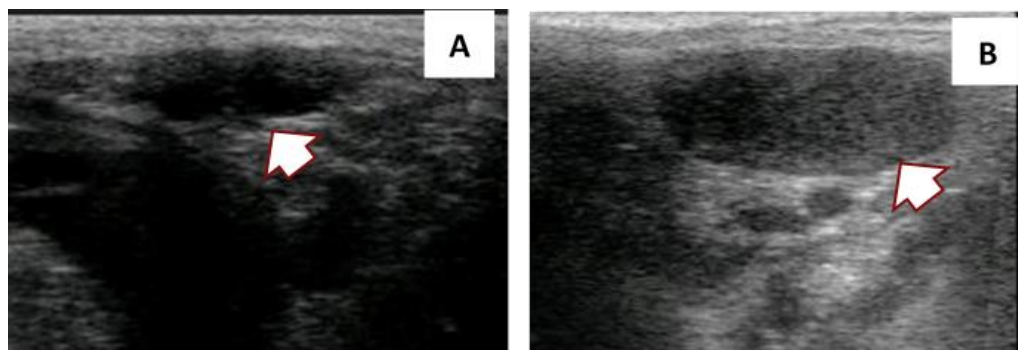
Figure 4.15: The ultrasonographic measurement of supramammary lymph nodes in goats: A) Normal, B) Clinical mastitis
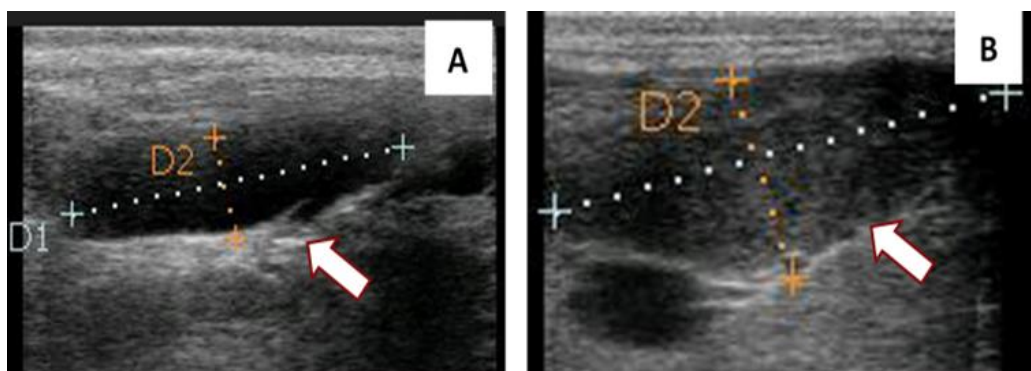
Figure 4.16: The ultrasonographic measurement of supramammary lymph nodes in cattle: A) Normal, B) Clinical mastitis

Table 4.4: The ultrasonographical measurement of supramammary lymph nodes in goats

Category	Right lymph node		Left lymph node	
	Length (mm) (mean ± SD)	Width (mm) (mean ± SD)	Length (mm) (mean ± SD)	Width (mm) (mean ± SD)
Normal (N=17)	15.0 ± 1.9^c	5.9 ± 0.9^c	14.7 ± 1.7^c	5.3 ± 0.9^c
Subclinical (N=13)	17.1 ± 2.1^b	7.0 ± 1.2^b	16.5 ± 2.0^b	6.2 ± 1.1^b
Clinical (N=10)	21.6 ± 2.2^a	9.6 ± 1.3^a	20.5 ± 2.6^a	9.1 ± 1.2^a

Different superscript letters a, b, c indicate statistically significant ($p<0.05$) within same column

Table 4.5: The ultrasonographical measurement of supramammary lymph nodes in cattle

Category	Right Lymph node		Left Lymph node	
	Length (mm) (mean ± SD)	Width (mm) (mean ± SD)	Length (mm) (mean ± SD)	Width (mm) (mean ± SD)
Normal (N=2)	52.5 ± 0.2^c	21.1 ± 1.2^c	51.1 ± 0.4^c	19.3 ± 0.9^c
Subclinical (N=3)	75.14 ± 4.2^b	26.9 ± 1.1^b	72.6 ± 2.0^b	26.2 ± 1.1^b
Clinical (N=5)	110.5 ± 6.1^a	29.8 ± 5.5^a	109.4 ± 3.4^a	29.5 ± 1.4^a

Different superscript letters a, b, c indicate statistically significant ($p<0.05$) within same column

4.4 Haematological examination

4.4.1 The measurement of haematological parameters in goats and cattle

The haematological parameters of normal, subclinical and clinical mastitis in goats and cattle are presented in Table 4.6.

The mean values of haemoglobin in normal goats, goats affected with subclinical mastitis and clinical mastitis were 8.8 ± 0.9 g/dl, 8.1 ± 0.5 g/dl and 7.5 ± 0.6 g/dl respectively. Comparatively, the values of Hb were significantly ($p<0.001$) different among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of hemoglobin was significantly different between normal and subclinical mastitis, and between normal and clinical mastitis in goats. However, there was no significant different between subclinical and clinical mastitis in goats.

The mean values of Hb in normal cattle, cattle affected with subclinical mastitis and clinical mastitis were 11.1 ± 0.2 g/dl, 9.1 ± 0.9 g/dl and 8.2 ± 0.8 g/dl respectively. The values of Hb were significantly ($p<0.005$) different among normal, subclinical and clinical mastitis in cattle. Although, the mean value of Hb was significantly higher in normal cattle than both the subclinical and clinical mastitis but there was no significant different between subclinical and clinical mastitis in cattle.

The mean values of packed cell volume (PCV) in normal goats, goats affected with subclinical mastitis and clinical mastitis were found $25 \pm 3.6\%$, $23.4 \pm 1.7\%$ and $22.6 \pm 1.9\%$ respectively, whereas the values of PCV in subclinical and clinical mastitis were less than the normal goats. Moreover, the values of PCV were significant different ($p<0.05$) among normal, subclinical and clinical mastitis in goats.

The mean values of packed cell volume (PCV) in normal cattle, cattle affected with subclinical mastitis and clinical mastitis were found $27.6 \pm 0.5\%$, $24.8 \pm 0.9\%$ and $23.8 \pm 1.3\%$ respectively. Although, the values of PCV were significant different ($p<0.05$) among normal, subclinical and clinical mastitis in cattle but in pairwise comparison, the values of PCV was significantly higher in normal cattle than clinical mastitis in cattle.

The mean values of total erythrocyte count (TEC) in normal goats, goats affected with subclinical mastitis and clinical mastitis were found 11.3 ± 2.0 million/µl, 9.1 ± 1.8 million/µl and 8.6 ± 2.0 million/µl respectively. The mean values of total erythrocyte

count (TEC) were significantly different ($p<0.001$) among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the mean value of TEC was significantly higher in normal goats than both the subclinical and clinical mastitis in goats although there was no significant different between subclinical and clinical mastitis in goats.

The mean values of total erythrocyte count (TEC) in normal cattle, cattle affected with subclinical mastitis and clinical mastitis were found 7.9 ± 0.5 million/µl, 7.1 ± 0.2 million/µl and 6.5 ± 0.6 million/µl respectively. The mean values of TEC were significantly different ($p<0.05$) among normal, subclinical and clinical mastitis in cattle. The mean value of TEC was significantly higher in normal cattle than the clinical mastitis in cattle however there was no significant different between normal and subclinical mastitis, and subclinical and clinical mastitis in cattle.

The mean values of total leukocyte count (TLC) in normal goats, goats affected with subclinical and clinical mastitis were 8.5 ± 1.9 thousand/µl, 8.9 ± 2.5 thousand/µl and 13.5 ± 3.5 thousand/µl respectively. The mean values of TLC were significantly different ($p< 0.001$) among normal goats, goats affected with subclinical and clinical mastitis. In pairwise comparison, the mean values of TLC in goats affected with clinical mastitis were significantly higher than both the subclinical mastitis and normal goats. However, there was no significant different between normal and goats affected with subclinical mastitis.

The mean values of total leukocyte count (TLC) in normal cattle, cattle affected with subclinical and clinical mastitis were 7.1 ± 0.2 thousand/µl, 8.5 ± 1.5 thousand/µl and 13.2 ± 1.1 thousand/µl respectively. The mean values of TLC were significantly different ($p< 0.001$) among normal cattle, cattle affected with subclinical and clinical mastitis. In pairwise comparison, the mean values of TLC in cattle affected with clinical mastitis were significantly higher than both the subclinical mastitis and normal cattle. However, there was no significant different between normal and cattle affected with subclinical mastitis.

Table 4.6: The measurement of haematological parameters in normal, subclinical and clinical mastitis in goats and cattle.

Parameters	Diagnosis status	Goat				Cattle			
		N	Mean ± SD	*p* value	Reference range	N	Mean ± SE	*p* value	Reference range
TEC (million/mcl)	Normal	17	11.3 ± 2.0^a	0.001	8-18	2	7.9 ± 0.5^a	0.02	5-10
	Subclinical	13	9.1 ± 1.8^{bc}			3	7.1 ± 0.2^{ab}		
	Clinical	10	8.6 ± 2.0^c			5	6.5 ± 0.6^b		
TLC (thousand/mcl)	Normal	17	8.5 ± 1.9^{bc}	0.001	4-13	2	7.1 ± 0.2^{bc}	0.001	4-12
	Subclinical	13	8.9 ± 2.5^b			3	8.5 ± 1.5^b		
	Clinical	10	13.5 ± 3.5^a			5	13.2 ± 1.1^a		
Hb (g/dl)	Normal	17	8.8 ± 0.9^a	0.001	8-12	2	11.1 ± 0.2^a	0.005	8-15
	Subclinical	13	8.1 ± 0.5^b			3	9.1 ± 0.9^b		
	Clinical	10	7.5 ± 0.6^{bc}			5	8.2 ± 0.8^{bc}		
PCV (%)	Normal	17	25 ± 3.6	0.05	22-38	2	27.6 ± 0.5^a	0.01	24-46
	Subclinical	13	23.4 ± 1.7			3	24.8 ± 0.9^{ab}		
	Clinical	10	22.6 ± 1.9			5	23.8 ± 1.3^b		
Lymphocyte (%)	Normal	17	53.2 ± 6.9^a	0.001	50-70	2	60.5 ± 0.7^a	0.001	45-75
	Subclinical	13	50.8 ± 9.3^{ab}			3	57.0 ± 2.0^{ab}		
	Clinical	10	36.1 ± 7.1^c			5	37.6 ± 6.5^c		

Parameter	Group	n	Value	p-value	Reference range	n	Value	p-value	Reference range
Monocyte (%)	**Normal**	17	1.4 ± 0.6	NA	0-4	2	1.5 ± 0.7	NA	2-7
	Subclinical	13	1.2 ± 0.7			3	1.5 ± 0.7		
	Clinical	10	1.9 ± 0.7			5	2.2 ± 0.8		
Neutrophil (%)	**Normal**	17	43.2 ± 6.6^c	0.001	30-48	2	36.5 ± 2.1^{bc}	0.005	15-45
	Subclinical	13	45.5 ± 8.8^{bc}			3	39.7 ± 1.5^b		
	Clinical	10	58.8 ± 12.2^a			5	56.2 ± 2.2^a		
Mature Neutrophil (%)	**Normal**	17	99.0 ± 0.9^a	0.001	99-100%	2	98.5 ± 0.7^a	0.005	99-100%
	Subclinical	13	97.7 ± 0.8^{ab}			3	97.3 ± 0.7^{ab}		
	Clinical	10	94.0 ± 2.9^c			5	94.6 ± 1.1^c		
Band cell (%)	**Normal**	17	1.0 ± 0.9^c	0.001	0-2%	2	1.5 ± 0.7^{bc}	0.005	0-2%
	Subclinical	13	2.3 ± 0.9^{bc}			3	2.7 ± 0.6^b		
	Clinical	10	6.0 ± 2.9^a			5	5.4 ± 1.1^a		
Eosinophil (%)	**Normal**	17	1.6 ± 1.0	NA	1-8	2	1.5 ± 0.7	NA	2-20
	Subclinical	13	2.1 ± 1.3			3	1.7 ± 0.6		
	Clinical	10	2.8 ± 1.5			5	2.4 ± 0.7		
Basophil (%)	**Normal**	17	0.6 ± 0.5	NA	0-1	2	0.5 ± 0.7	NA	0-2
	Subclinical	13	0.5 ± 0.5			3	0.7 ± 0.6		
	Clinical	10	0.7 ± 0.6			5	1.0 ± 0.7		

Different superscript letters a, b, c indicate statistically significant difference ($p<0.05$) within same column, NA indicates non-significant.

Reference range: (D'Andrea and Sjogren, 2014)

The mean values of total leukocyte count (TLC) in normal cattle, cattle affected with subclinical and clinical mastitis were 7.1 ± 0.2 thousand/µl, 8.5 ± 1.5 thousand/µl and 13.2 ± 1.1 thousand/µl respectively. The mean values of TLC were significantly different ($p < 0.001$) among normal cattle, cattle affected with subclinical and clinical mastitis. In pairwise comparison, the mean values of TLC in cattle affected with clinical mastitis were significantly higher than both the subclinical mastitis and normal cattle. However, there was no significant different between normal and cattle affected with subclinical mastitis.

In the differential leukocyte count, the mean value of neutrophils in normal, subclinical and clinical mastitis were 43.2 ± 6.6%, 45.5± 8.8% and 58.8 ± 12.2% respectively which were significantly different ($p < 0.001$) among the three groups. In pairwise comparison, the mean value of neutrophils in goats affected with clinical mastitis was significantly higher in both the goats affected with subclinical mastitis and the normal goats. However, there was no significant different between the goats affected with subclinical mastitis and the normal goats.

In cattle, the mean value for neutrophils in normal, subclinical and clinical mastitis were 36.5 ± 2.1%, 39.7± 1.5% and 56.2 ± 2.2% respectively which were significantly different ($p < 0.001$) among the three groups. The mean value of neutrophils in cattle affected with clinical mastitis was significantly higher in both the cattle affected with subclinical mastitis and the normal goats. However, there was no significant different between the cattle affected with subclinical mastitis and the normal goats.

On observing the type of neutrophils under the microscope, the percentages of mature neutrophils (segmented nucleus) in normal goats, goats affected with subclinical and clinical mastitis were found 99.0 ± 0.9%, 97.7 ± 0.8% and 94.0 ± 2.9% respectively which were significantly different ($p < 0.001$) among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the percentage of mature neutrophils was significantly higher in normal goats than goats affected with clinical mastitis, and was also significantly higher in subclinical mastitis than the clinical mastitis in goats. However, there was no significant different between normal and goats affected with subclinical mastitis. On the other hand, the percentages of band cells (immature neutrophils) in normal goats, goats affected with subclinical and clinical mastitis were found 1.0 ± 0.9%, 2.3 ± 0.9% and 6.0 ± 2.9% respectively which were significantly

different ($p<0.001$) among normal, subclinical and clinical mastitis in goats. In pairwise comparison, the percentage of band cells was significantly higher in cattle affected with clinical mastitis than both subclinical mastitis and normal goats. However, there was no significant different between the normal goats and the goats affected with subclinical mastitis.

In cattle, the percentages of mature neutrophils (segmented nucleus) in normal cattle, cattle affected with subclinical and clinical mastitis were found 98.5 ± 0.7%, 97.3 ± 0.7% and 94.6 ± 1.1% respectively which were significantly different (P<0.005) among normal, subclinical and clinical mastitis in cattle. In pairwise comparison, the percentage of mature neutrophils was significantly higher in normal cattle than the cattle affected with clinical mastitis, and was also significantly higher in subclinical mastitis than the clinical mastitis in cattle. However, there was no significant different between the normal cattle and the cattle affected with subclinical mastitis. On the other hand, the percentages of band cells (immature neutrophils) in normal cattle, cattle affected with subclinical and clinical mastitis were found 1.5 ± 0.7%, 2.7 ± 0.6% and 5.4 ± 1.1% respectively which significantly different ($p<0.005$) among normal, subclinical and clinical mastitis in cattle. In pairwise comparison, the percentage of band cells was significantly higher in cattle affected with clinical mastitis than both subclinical mastitis and normal cattle. However, there was no significant different between the normal cattle and cattle affected with subclinical mastitis.

The mean values of percentage of lymphocytes in normal goats, goats affected with subclinical and clinical mastitis were 53.2 ± 6.9%, 50.8 ± 9.3% and 36.1 ± 7.1% respectively which were significantly different ($p< 0.001$) among the three groups. In pairwise comparison, the mean value of the percentage of lymphocytes in normal goats was significant higher than the clinical mastitis in and was also significantly higher in subclinical mastitis than the clinical mastitis in goats. However, there was no significant different between the normal goats and goats affected with subclinical mastitis.

The mean value of percentage of lymphocytes in normal cattle, cattle affected with subclinical and clinical mastitis were 60.5 ± 0.7%, 57.0 ± 2.0% and 37.6 ± 6.5% respectively which were significantly different ($p< 0.001$) among the three groups. In

pairwise comparison, the mean value of the percentage of lymphocytes in normal cattle was significant higher than the clinical mastitis in and was also significantly higher in subclinical mastitis than the clinical mastitis in cattle. However, there was no significant different between the normal cattle and the cattle affected with subclinical mastitis.

The mean value of percentage of monocytes, eosinophils and basophils in normal goats were found $1.4 \pm 0.6\%$, $1.6 \pm 1.0\%$, and $0.6 \pm 0.5\%$ respectively. The mean value of percentage of monocytes, eosinophils and basophils in goats affected with subclinical mastitis were found $1.2 \pm 0.7\%$, $2.1 \pm 1.3\%$, and $0.5 \pm 0.5\%$ respectively. The mean value of percentage of monocytes, eosinophils and basophils in goats affected with clinical mastitis were found $1.9 \pm 0.7\%$, $2.8 \pm 1.5\%$, and $0.7 \pm 0.6\%$ respectively. The mean value of percentage of monocytes, eosinophils and basophils were not significantly different among normal, subclinical and clinical mastitis in goats.

In normal cattle, the mean value of percentage of monocytes, eosinophils and basophils were found $1.5 \pm 0.7\%$, $1.5 \pm 0.7\%$, and $0.5 \pm 0.7\%$ respectively. The mean value of percentage of monocytes, eosinophils and basophils in cattle affected with subclinical mastitis were found $1.5 \pm 0.7\%$, $1.7 \pm 0.6\%$, and $0.7 \pm 0.6\%$ respectively. The mean value of percentage of monocytes, eosinophils and basophils in goats affected with clinical mastitis were found $2.2 \pm 0.8\%$, $3.4 \pm 0.7\%$, and $1.0 \pm 0.7\%$ respectively. Although, the mean value of percentage of monocytes and basophils were not significantly different among normal, subclinical and clinical mastitis in cattle, interestingly the mean value of percentage of eosinophils was significantly different ($p<0.05$) among normal, subclinical and clinical mastitis in cattle.

Chapter - 5: Discussion

The practicality of using ultrasonography as a non-invasive diagnostic tool for detection of pathological conditions of mammary gland in animals by European standards. As susceptibility to infection is a factor attributed primarily to the anatomy and functioning of the udder and teats, was discussed in our study about the values of clinical examinations and ultrasonography for measuring structures and conditions of the udder and teats in ruminants.

In the present study, 40 lactating goats of Jamnapari crossbred were examined clinically whereas SCM was estimated 32% (13/40) based on the DMSCC which is supported by previous studies in Bangladesh publishing 36% (Islam et al., 2012), 38.75% (Ferdous et al., 2018), 37.2% (Amin et al., 2017) SCM were found in goats. However, the percentage of SCM was lower in our study than the prevalence (50%) was found in another study conducted in Chattogram metropolitan area by CMT (Akter et al., 2020). Besides, the lower prevalence of SCM was reported in a number of studies such as 18.29% (Rizwan et al., 2016), 18.64% (Razi et al., 2012), 24.6% (Roukbi et al., 2015) in Bangladesh and other countries such as 22.5% in Brazil 22.5% (Schmidt et al., 2009), 13% in Pakistan (Ali et al., 2010) and 18.03% in Ethiopia. CM in goats was noticed 25% (10/40) in our study which was higher than the previous studies reporting 11.67% (Ferdous et al., 2018), 6% (Islam et al., 2012) and not more than 5% was reviewed by Contreras et al. (2007). This may be due to the influence of multiple factors such as breed, age and litter size, lactation period and body score condition, different management practices followed on each farm (Contreras et al., 1995; Boscos et al., 1996 and McDougall et al., 2002). In quarter levels, left quarters were more affected with mastitis compare to right quarters which is supported by previous study where highly positive (+++) CMT reactions were recorded for 47% (14/30) left halves and 30% (9/30) right halves (Franz et al., 2009). Sarker and Samad et al. (2011) reported the prevalence of clinical mastitis was found higher in left udder-haves (79.66%) in comparison to the right udder-halve (20.34%). On the other hand, Radostits et al. (2007) stated that the prevalence of intra mammary infection is about 50% in cattle which is similar with our findings where the percentage of CM was 50% in cattle. Besides, the percentage of subclinical mastitis in

cow was 30% in this study which is consistent with previous study where 33.56% dairy cattle affected with subclinical mastitis based on CMT in Chattogram district of Bangladesh (Barua et al., 2014).

On clinical examination of udder in goats, both the SCM and CM were found more frequent in asymmetrical udders (17.5%, 7/40 and 17.5%, 7/40) compared to symmetrical udders (15%, 6/40 and 7.5% (3/40), which is agreed with Margatho et al. (2020) reported that asymmetrical udders were found higher SCC than symmetrical udders in goats. In the present study, pendulous shaped udders (27.5%, 11/40 and 17.5%, 7/40) were more affected with both the SCM and CM than spherical-shaped udders (5%, 2/40) and 7.5%, 3/40) which is supported by previous study where the SCC was higher in pendulous shaped udder than spherical-shaped udder (Montaldo and Martinez-Lozano, 1993; Margatho et al., 2020). On the other hand, bottle and cylindrical shaped teats were more prone to mastitis than funnel shaped teats in goats which is consistent with previous studies where the prevalence of mastitis in goats and sheep were more frequent in cylindrical and bottle shaped teats (James et al., 2009; Akter et al., 2020). During the evaluation of udder, visible abnormalities were observed in clinical mastitis such as swollen of udder, induration, painful on palpation, warm on touch and changes of color of udder's skin (erythema and bluish discoloration) which have been also reported by previous studies (Contreras et al., 2007, Paterna et al., 2014, Machado, 2018; Awad et al., 2008 and Singh et al., 2018).

In the current study, milk abnormalities such as white clotted, bloody and watery milk were found in CM in goats and cattle which have been also reported by previous studies (Contreras et al., 2007, Paterna et al., 2014, Machado, 2018; Awad et al., 2008 and Singh et al., 2018).

The physical characteristics of the mammary gland have been the subject of comprehensive research. The teat and natural defense barriers of the teat canal against pathogens are of central importance. The ultrasound measurement of mammary gland structures showed statistically significant differences ($p<0.001$) in teat canal length, teat canal diameter and teat wall thickness among normal, SCM and CM in goats in the present study. We found that teat canal length and teat canal diameter were significantly ($p<0.01$) shorter and narrower in normal animal than both the SCM and CM in goats. Besides, the teat canal length and teat canal diameter were shorter and

narrower in normal animal than both the SCM and CM in cattle without statistically significant differences. The influence of length and diameter of teat canals on the incidence of bacterial infection has been focused on previous studies by McDonald (1975), Grindal and Hillerton (1991), Scherzer (1997), Franz et al. (2009) and Fasulkov et al. (2015), Amin et al. (2017). Of these, Amin et al. (2017) suggested that teat canal length was significantly shorter in normal cows than affected cows, and teat canal length and teat canal diameter were decreased in experimental affected goats after 72 hours than prior to infection but increased teat canal length after infection at168 hours by Fasulkov et al. (2015) which is contradictory of our present study. On the other hand, longer and wider teat canal were significantly highly positive to the CMT in ewes which is supported to our study (Franz et al., 2009). Scherzer (1997) postulated that the length of the teat canal had no association with the incidence of acute mastitis, but a longer teat canal provided more space for the establishment of pathogens, which may be related to chronic or subclinical mastitis. Grindal and Hillerton (1991) stated that the length of the teat canal influenced on new infection which was not significant. McDonald (1975) stated that a shorter teat canal provided more resistance to infection, albeit a significant relationship was not evident. . Albeit, teat wall thick ness was significantly increased in CM than both the SCM and normal goats, but there was no significant evident in cattle. The thickness of teat wall was significantly increased in experimental goats after infection at 96 hours in accordance with our study (Fasulkov et al., 2015). Previous studies stated that the teat wall thickness was increased due to response to the inflammation of the teat wall mucosa, proliferative growth of the mucosa and fibrosis due to chronic infection (Fasulkov et al., 2015; Edwards et al., 2000; Fasulkov et al., 2014 and Couture and Mulon., 2005). Amin et al. (2017) stated that the thickness of teat wall was increased in cattle and buffalo affected with mastitis than normal animals without any significant evident. The teat cistern diameter was not significantly different among normal, subclinical and clinical mastitis in both the goats and cattle which is supported by previous studies (Amin et al., 2017 and Fasulkov et al., 2015).

The echogenicities of mammary gland parenchyma were homogeneous and hypoechoic in normal goats and cattle whereas heterogeneous and hyperechogenicity were found in CM in goats and cattle in the present study. The findings are similar with previous studies where normal mammary gland parenchyma was homogeneous

and hypoechoic and observed non-homogenous and hyperechogenicity in acute and chronic mastitis (Fasulkov et al., 2014, Trostle and O'Brien, 1998, Hussein et al., 2015; Banting, 1998). In contrast, some reports stated that normal mammary parenchyma in cattle and buffalo is appeared as homogeneous and hyperechoic (Rambabu et al., 2008; Ayadi et al., 2003). Hyperechogenicity can be explained by the inflammatory process that results in tissue fibrosis, which has higher density than normal mammary parenchyma. As inflammation causes changes in echogenicity of affected organs, quantitative evaluation could be used to assess inflammation by attributing a numerical value to echogenicity (Santos et al., 2014). Interestingly, 5% (2/40) quarters (right and left) in goats and 7.5% (3/40) quarters in cattle were found heterogeneous due to hyperechoic spots in mammary parenchyma in case of SCM. Hussein et al (2015) stated that the echogenicity of mammary parenchyma was not changed between normal and subclinical mastitis in ewes, however, one infected half had heterogeneous echogenicity.

In the current study, the lactiferous ducts in udder parenchymal tissues were significantly clearly visible in normal and SCM whereas the lactiferous ducts were hardly visible in CM in both the goats and cattle which are similar with previous studies (Fasulkov et al., 2014; Santos et al., 2014 and Franz et al, 2009). Mourya et al (2020) claimed that mammary parenchyma of cattle revealed positive for subclinical mastitis by Modified CMT appeared with lack of clarity of visualization of milk alveoli and lactiferous duct which is contradictory to our study. The lactiferous ducts in CM are hardly visible due to milk alveoli contained hypo to hyperechoic suspended flakes (Kotb et al, 2014; Amin et al., 2017). Fasulkov et al. (2015) stated that the diameter of lactiferous ducts in the parenchyma became narrow after inoculation at 72 hours, it became even narrower at 168 hours than the initial value due to accumulation of exudates.

The contents of gland and teat cisterns were found anechoic echogenicity in normal and SCM, although hypoechoic and/or hyperechoic contents were found in CM in both the goats and cattle which were significantly different among three groups. The findings are similar with previous studies where gland and teat cisterns were found hypo to hyperechoic structures in clinical mastitis in both goats and cattle (Amin et al., 2017, Fasulkov et al., 2015; Santos et al., 2014). The presence inflammation was

clearly indicated by imaging of hypo to hyperechoic structures in the gland and teat cistern representing milk coagula after milking (Fasulkov et al., 2015).

Although regular and irregular lining of teat wall were found in both the normal and SCM but irregular lining of teat wall was found in clinical mastitis in both the goats and cattle. Moreover, the contour of lining of teat wall was significantly different among normal, SCM and CM in both the ruminants. The irregular contour of teat sinus and absence of the three layered appearance of teat were found in minimum inflammation with subclinical mastitis in cattle and Buffalo (Dine et al., 2000; Kotb et al., 2014; Szenziova and Strapak, 2012 and Abd Al-Galil and Khalil, 2016; Mourya et al., 2020) which are similar with our study. Gleeson et al (2004) stated that teat tissue changes were found in ultrasonography in normal cows due to different milking systems. The irregularity of teat wall lining was found in clinical mastitis in goats, sheep, cattle and buffalo due to separation of outer layer of teat wall, abscessation, proliferation of teat wall, inflamed teat wall and longitudinal caseated materials below the mucosa of the teat cistern upholding the present study (Amin et al., 2017, Mavrogianni et al., 2004, Fasulkov et al., 2014; John et al., 1998, Fasulkov et al., 2015; Santos et al., 2014). Teat canal obstruction was imaged in CM in both the goats (2.5 to 5%) and cattle (12.5%) which is supported by previous studies (Amin et al., 2017; Kotb el al., 2014, Franz et al., 2009). The obstruction of the teat canal in CM was occurred owing to hyperechoic caseated materials in teat cistern, fibrosis and proliferation of granulation tissues of rosette of Furstenberg leading impaired milk secretion (Amin et al., 2017; Riedl et al., 2004).

The length and width of supramammary lymph nodes in CM were significantly more increased than the SCM and normal goats and cattle, even increased in subclinical mastitis than the normal animals, which is supported by previous studies (Dohoo and Leslie 1991; Bradley et al., 2001; Soltys and Quinn, 1999; Khoramian et al., 2015; Risvanli et al., 2019). Hussein et al. (2015) stated that the ultrasonographic measurement of length, depth and area of the supramammary lymph nodes were significantly ($p<0.05$) increased in infected udder whereas sensitivity of measurement of lymph node to subclinical mastitis was 96, 92 and 94% respectively, in contrast the sensitivity of SCC was 94% and CMT was 68.8%. During mastitis in animals, the lymphocytes in the supramammary lymph nodes of the infected quarter are rapidly activated, proliferate and migrate to the mammary gland to fight bacterial infection,

and also expected that supramammary lymph nodes have different size and architecture according to the infectious status of the animals (Soltys and Quinn, 1999; Bradley et al., 2001; Kehrli and Harp, 2001). Moreover, Khoramian et al. (2015) noticed that ultrasonography was a useful technique for detecting morphological changes in supramammary lymph nodes in dairy cows.

In the present study, the mean value of haemoglobin in goats and cattle was significantly higher in normal animal than both the SCM and CM in animals but there was no significant different between SCM and CM. The findings are in accordance with previous studies where average Hb were statistically higher in healthy animals than those with SCM in goats (Hristov et al., 2018), and SCM and CM in cattle (Das et al., 2018). Author reported that there were no significant changes in mean value of Hb between SCM and CM in cattle which is consistent to the present study (Das et al., 2018).

Besides, the values of PCV were significantly higher in both the normal goats and cattle than the CM which is supported by many published reports (Das et al., 2018; Abba et al., 2013). Other studies stated that PCV did not show significant differences between healthy animals and animals affected with subclinical mastitis in accordance with our study (Hristov et al., 2018; Hussein et al., 2016).

In the present study, the mean value of TEC was significantly higher in normal goats than both the SCM and CM in goats which is supported by previous published reports where erythrocytes counts are significantly increased in healthy animals than the SCM and CM in goats (Ajuwape et al., 2005; Abba et al., 2013; Hristov et al., 2018). Although the mean value of TEC was significantly higher in normal cattle than the clinical mastitis, interestingly there was no significant different between normal and SCM in this study. Das et al. (2018) stated that TEC in healthy cows was significantly higher than the cows affected with CM but no significant change was observed between SCM and CM in animals. In contrast, TEC in healthy cows was significantly higher than the cows affected with SCM but no significant change was observed between healthy and animals affected CM (Sarvesha et al., 2017). However, Hb, PCV and TEC levels in healthy cows were not significantly different with cows affected with CM reported by Sischo et al. (1997) which is inconsistent with this study. The variations might be explained by the influence of many additional factors such as

nutrition, habitat, environment, age, reproductive status and stress of the animals. (Zumbo et al., 2011; Waziri et al., 2010).

In the present study, the mean values of TLC in both the goats and cattle affected with CM were significantly higher than the normal animals, which is supported by many previous studies (Das et al., 2018; Abba et al., 2013; Ajuwape et al., 2005). Interestingly, no significant different was observed between the normal goats and cattle, and animals affected with SCM in this study. Dang et al. (2007) and Khaled et al. (2015) observed no significant changes in TLC between the healthy animals and animals affected with subclinical mastitis because the fact is that systemic reaction is not occurred as the infection is localized in mammary gland which is similar with our study. In contrast, authors observed a significant increase of TLC in animals affected with SCM compared to healthy animals due to invasion and spread of pathogens in udder and systemic reaction of the body. (Hussien et al., 2015; Sarvesha et al., 2017; Hristov et al., 2018)

The percentage of neutrophils in both goats and cattle affected with CM were significantly higher in normal animals and animals affected with subclinical mastitis which is consistent with previous studies describing during inflammation, neutrophil recruitment is increased at site of infection because of chemotactic factors which are released by infectious agents and other immune system components (Abba et al., 2013; Hussien et al., 2015; Sarvesha et al., 2017; Das et al., 2018 and Saleh et al., 2022). A significant percentage of mature neutrophils was higher in both the normal goats and cattle than the animals affected with CM, and was significantly higher in SCM than CM in animals in the present study. Besides, we also observed the percentage of band cells (immature neutrophils) was significantly higher in both goats and cattle affected with CM than the SCM and healthy animals which is supported by Hussien et al. (2015). It might be occurred due to more neutrophils shift to the udder to fight against infection where bone marrow releases more immature neutrophils which were band shaped.

In the present study, the percentage of lymphocytes in both normal goats and cattle were significantly higher than animals affected with CM and were also significantly higher in SCM than CM in animals which are consistent with previous studies where lymphopenia was found in clinical mastitis (Hussien et al., 2015; Sarvesha et al.,

2017; Das et al., 2018). Although, the percentage of monocyte was increased in animals affected with clinical mastitis than normal animals but authors didn't observe any significant difference which is similar with our current study (Das et al., 2018; Sarvesha et al., 2017). However, Ajuwape et al. (2005) significantly observed monocytosis in clinical mastitis than the normal animals due to acute tissue necrosis and endotoxin associated with septic mastitis which is in contrast to the present study.

Moreover, fast and accurate diagnostics and prognosis are very important with regard to mammary gland illness in ruminants due to the negative economic impact on the loss of milk productivity. This necessitates the usage of modern, accurate and quick methods for mammary gland examination, such as ultrasonography. Possible indications for application of ultrasound could be disruptions in milk secretion, diagnostics of pathological changes (stenosis and obstructions, inflammations, abscesses, haematomas, foreign bodies etc.), which cannot be easily detected through clinical examination or others diagnostic aids. As it determines the dimension of structures within the area of the teat, it could be useful in guiding the prevention of mastitis in ruminants because teat parameters are also anatomical and functional risk factors for the development of mastitis.

Chapter - 6: Conclusions

In this study, asymmetrical udders were found to be more prone to both subclinical and clinical mastitis than symmetrical udders, and pendulous shaped udders were more affected with subclinical and clinical mastitis than spherical-shaped udders in goats. Besides, bottle and cylindrical-shaped teats were more susceptible to subclinical and clinical mastitis than funnel-shaped teats in goats. During clinical mastitis in goats and cattle, visible abnormalities in the udder and teat, such as swelling, induration, pain on palpation, warm on touch, and changes in skin color were observed, as well as milk abnormalities such as white clotted, bloody, and watery milk were found.

In the current study, teat canal length, teat canal diameter, and teat wall thickness were significantly different among normal, subclinical, and clinical mastitis in goats, but there was no significant difference in cattle. In clinical mastitis, the mammary parenchyma was non-homogeneous and hypo to hyperechoic, with a lack of clear visualization of the lactiferous ducts and gland cisterns were hypo to hyperechoic contents, whereas in healthy udder, the mammary parenchyma was homogeneous and hypoechoic, and the gland cistern was anechoic. When the goats and cattle affected with clinical and subclinical mastitis were compared to normal animals, the ultrasonographical measurement of length and width of the supramammary lymph nodes were significantly increased.

The haemoglobin, packed cell volume and total erythrocyte count were significantly different among normal, subclinical and clinical mastitis in both goats and cattle. Besides, total leukocyte count, the percentage of neutrophils and band cells were significantly increased in clinical mastitis than the normal and subclinical mastitis in both the goats and cattle. The haematological counts could be a true reflection of an animal's health status.

The clinical and ultrasonographical evaluations accurately reflect mastitis and can even help clinicians predict the prognosis of animals affected with mastitis. In the future, ultrasonography may be used as a quick complementary technique in the field practices to diagnose the mastitis in ruminants.

Chapter - 7: Limitations and Recommendations

Limitations:

Small sample size of this investigation was not representative to the population due to short period of the study. Lacking of several reliable diagnostic methods like bacteriological culture, automatic counting of somatic cells by flow cytometry, multiplex-PCR and specific enzymes indicator of breast tissue lesion such as NAGase for investigating the mastitis.

Recommendations:

Though a significantly positive conclusion was found in this study, however, large sized population will provide more specified result for better conclusion. So, it is suggested that combining of several diagnostic protocol will confirm the diagnosis of mastitis. There was a single measurement of teat parameters taking on sonogram, therefore reproducibility of measurement of ultrasound will help in order to confirm the precise results.

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Appendix – I

A Questionnaire for Clinical examination of Goats and Cattle as well as Mammary gland

Patient ID: Mb no.

Breed: Age: Body weight: Calving time:

Parity:

Onset of illness:

Clinical findings:

Basic parameters:

General appearance:

Temperature: Heart rate: Respiration rate:

Mucous membrane:

Udder Examination:

Shape: Symmetry:

Inspection: Palpation:

Teat Examination: Shape: Symmetry:

Lymph node examination:

Inspection:

Palpation:

Ultrasonographic Measurement of Mammary gland in Goats

Traits		Right Quarter	Left Quarter
Teat Canal Length (mm)	Any clinical findings		
Teat Canal Diameter (mm)	Any clinical findings		
Teat Wall Thickness (mm)	Echogenesity Lining Findings		
Teat cistern Diameter (mm)	Echogenesity Findings		
Udder parenchyma	Echogenesity Findings Lactiferous Ducts Gland cistern		
Lymphnode			
Length (mm)	Echogenesity Findings		
Width (mm)	Echogenesity Findings		

Ultrasonographic Measurement of Mammary gland in Cattle

Traits		Right fore quarter	Left fore quarter	Right hind Quarter	Left hind quarter
Teat Canal Length (mm)	Any clinical findings				
Teat Canal Diameter (mm)	Any clinical findings				
Teat Wall Thickness (mm)	Echogenesity Lining Findings				
Teat cistern Diameter (mm)	Echogenesity Findings				
Udder parenchyma	Echogenesity Findings Lactiferous Ducts Gland cistern				
Lymph nodes					
Length (mm)	Echogenesity Findings				
Width (mm)	Echogenesity Findings				

Appendix – II

Laboratory Analysis

The Formula of Counting of Somatic Cell in Milk by DMSCC Method

Calculation of Somatic Cell in milk

Calculate the total concentration, C of cells by using the following equation:

$$C = \frac{W_s \times L_s \times N_t}{\pi \times (\frac{D_f}{2})^2 \times N_f \times V_m} \times \frac{1}{d}$$

Or, $C = f_w \times [\frac{N_t}{N_f} \times \frac{1}{d}]$

Or, with the constant working factor, f_w

$$f_w = \frac{W_s \times L_s}{\pi \times (\frac{D_f}{2})^2 \times V_m}$$

Where, C is the total concentration, expressed in number of cells per millilitre;

W_s is the width in millimetres of the smear;

L_s is the length in millimetres, of the smear;

N_t is the total number of cells counted;

D_f is the diameter in millimetres of the microscope field;

N_f is the number of fields counted completely;

V_m is the volume in millilitres of the test sample smeared;

d is the dilution factor (If no dilution is required, d = 1).
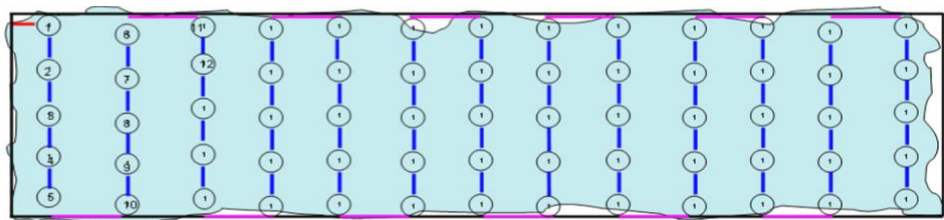
Counting of Microscopic Field on Stained Smear on 40X Magnification

Hematology Profile

Name of the Tests	Results	Normal Range
Total Count of TEC		
Total Count of TLC		
Hb (%)		
PCV (%)		
DLC ⇩		
Lymphocyte (%)		
Monocyte (%)		
Neutrophil (%)		
Eosinophil (%)		
Basophil (%)		

Appendix – III (A)

The ultrasonographical findings of the udder parenchyma and teat in goats. Pearson Chi-squared analysis was used to compare proportions and level of significance (p<0.05)

Traits	Right quarter (N=40)				Left quarter (N=40)			P- value
	Ultrasound findings	Normal (N=22)	Subclinical mastitis (N=11)	Clinical mastitis (N=7)	Normal (N=18)	Subclinical mastitis (N=14)	Clinical mastitis (N=8)	
Echotexture of udder parenchyma	Homogenous	55% (22/40)	22.5% (9/40)	0.00%	45% (18/40)	30% (12/40)	0.00%	0.01
	Heterogenous	0.00%	5% (2/40)	17.5% (7/40)	0.00%	5% (2/40)	20% (8/40)	
Hyperechoic spot on udder parenchyma	Yes	55% (22/40)	22.5% (9/40)	0.00%	45% (18/40)	30% (12/40)	0.00%	0.01
	No	0.00%	5% (2/40)	17.5% (7/40)	0.00%	5% (2/40)	20% (8/40)	
Visualization of lactiferous ducts	Clearly visible	55% (22/40)	27.5% (11/40)	0.00%	45% (18/40)	35% (14/40)	0.00%	0.01
	Hardly visible	0.00%	0.00%	17.5% (7/40)	0.00%	0.00%	20% (8/40)	
The contents of gland cistern	Anechoic	55% (22/40)	27.5% (11/40)	0.00%	45% (18/40)	35% (14/40)	0.00%	0.01
	Hypo to hyperechoic	0.00%	0.00%	17.5% (7/40)	0.00%	0.00%	20% (8/40)	
The contour of Teat wall	Regular	45% (18/40)	15% (6/40)	0.00%	37.5% (15/40)	25% (10/40)	5% (2/40)	0.05
	Irregular	10% (4/10)	12.5% (5/40)	17.5% (7/40)	7.5% (3/10)	10% (4/40)	15% (6/40)	
The contents of teat cistern	Anechoic	55% (22/40)	27.5% (11/40)	0.00%	45% (18/40)	35% (14/40)	0.00%	0.01
	Hypo to hyperechoic	0.00%	0.00%	17.5% (7/40)	0.00%	0.00%	20% (8/40)	

Appendix – III (B)

The ultrasonographical findings of the udder parenchyma and teat in cattle. Pearson Chi-squared analysis was used to compare proportions and level of significance (p<0.05)

Traits	Total quarter (N=40)				p- value
	Ultrasound findings	Normal (N=10)	Subclinical mastitis (N=19)	Clinical mastitis (N=11)	
Echotexture of udder parenchyma	Homogenous	25% (10/40)	40% (16/40)	0.00%	0.01
	Heterogenous	0.00%	7.5% (3/40)	27.5% (11/40)	
Hyperechoic spot on udder parenchyma	Yes	25% (10/40)	40% (16/40)	0.00%	0.01
	No	0.00%	7.5% (3/40)	27.5% (11/40)	
Visualization of lactiferous ducts	Clearly visible	25% (10/40)	47.5% (19/40)	0.00%	0.01
	Hardly visible	0.00%	0.00%	27.5% (11/40)	
The contents of gland cistern	Anechoic	25% (10/40)	47.5% (19/40)	0.00%	0.01
	Hypo to hyperechoic	0.00%	0.00%	27.5% (11/40)	
The contour of Teat wall	Regular	17.5% (7/40)	25% (10/40)	0.00%	0.05
	Irregular	7.5% (3/40)	22.5% (9/40)	27.5% (11/40)	
The contents of teat cistern	Anechoic	25% (10/40)	47.5% (19/40)	0.00%	0.01
	Hypo to hyperechoic	0.00%	0.00%	27.5% (11/40)	

Appendix – III (C)

<u>**The relationship of shape, symmetry of udder and teats, and mastitis in goats. Pearson Chi-squared analysis was used to compare proportions and level of significance (p<0.05)**</u>

Traits	Goats				p- value
	Category	Normal (N=17)	Subclinical mastitis (N=13)	Clinical mastitis (N=10)	
Shape of udder	Spherical	27.5% (11/40)	5% (2/40)	7.5% (3/40)	0.05
	Pendulous	15% (6/40)	27.5% (11/40)	17.5% (7/40)	
Shape of teat	Funnel	25% (10/40)	5% (2/40)	2.5% (1/40)	0.05
	Cylindrical	10% (4/40)	10% (4/40)	5% (2/40)	
	Bottle	7.5% (3/40)	17.5% (7/40)	17.5% (7/40)	
Symmetry of udder	Yes	32.5% (13/40)	15% (6/40)	7.5% (3/40)	0.05
	No	10% (4/40)	17.5% (7/40)	17.5% (7/40)	
Symmetry of teat	Yes	30% (12/40)	17.5% (7/40)	2.5% (1/40)	0.05
	No	12.5% (5/40)	15% (6/40)	22.5% (9/40)	

Biography

Thomby Paul is a candidate for the degree of MS in Surgery under the Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University (CVASU). He passed the Secondary School Certificate Examination in 2010 followed by Higher Secondary Certificate in 2012. He has obtained his Doctor of Veterinary Medicine (DVM) Degree in 2019 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. During his undergraduate period, he has received clinical training on Veterinary Medicine from Tamil Nadu Veterinary and Animal Sciences University, India. During his postgraduate period, he has received hands-on clinical training on advanced diagnostic imaging from West Bengal University of Animal and Fishery Sciences, India. He has achieved Dean's award for his academic excellence and also achieved "Best Clinical Award" in clinical practices from Teaching Veterinary Hospital, Chattogram Veterinary and Animal Sciences University. He has published several scientific articles in national and international journals. He has great interest on diagnostic imaging, small and large animal surgery.