



**PREVALENCE, ASSOCIATED RISK FACTORS, AND ISOLATION OF MAJOR
BACTERIAL CAUSES OF MASTITIS IN LACTATING COWS IN DARA
DISTRICT SIDAMA REGIONAL-STATE, ETHIOPIA**

MSc. THESIS

BY

ZERIHUN SHABULA

HAWASSA UNIVERSITY FACULTY OF VETERINARY MEDICINE

HAWASSA, ETHIOPIA

NOVEMBER, 2023

**PREVALENCE, ASSOCIATED RISK FACTORS, AND ISOLATION OF MAJOR
BACTERIAL CAUSES OF MASTITIS IN LACTATING COWS IN DARA
DISTRICT SIDAMA REGIONAL-STATE, ETHIOPIA**

MSc. THESIS

BY

ZERIHUN SHABULA

MAJOR ADVISOR: GIZACHEW HAILEGEBREAL (Asst. Professor)

CO-ADVISOR: MESELE ABERA (Assoc. Professor)

**A THESIS SUBMITTED TO HAWASSA UNIVERSITY FACULTY OF
VETERINARY MEDICINE, IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN VETERINARY EPIDEMIOLOGY**

HAWASSA, ETHIOPIA

NOVEMBER, 2023

ADVISORS' APPROVAL SHEET
SCHOOL OF GRADUATE STUDIES
HAWASSA UNIVERSITY ADVISORS' APPROVAL SHEET
(Submission Sheet-1)

This is to certify that the thesis entitled “Prevalence, associated risk factors and isolation of major bacterial causes of bovine mastitis in lactating cows at Dara district Sidama Regional-State, Ethiopia” submitted in partial fulfillment of the requirements for the degree of Master's with specialization in Veterinary Epidemiology, the Graduate Program of the Faculty of Veterinary Medicine, and has been carried out by **Zerihun Shabula**, ID. No. EpVePR 0012/14, under our supervision.

Therefore, we recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

_____	_____	_____
Name of major advisor	Signature	Date
_____	_____	_____
Name of co-advisor	Signature	Date

TABLE OF CONTENTS

TABLE OF CONTENTS	iv
ACKNOWLEDGMENTS	vi
DEDICATION	vii
STATEMENT OF THE AUTHOR	viii
LIST OF ABBREVIATIONS	ix
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ANNEXES	xii
ABSTRACT	xiii
1. INTRODUCTION	1
1.1. Statement of the problem	3
1.2. Objectives of the study	4
1.2.1. General objective	4
1.2.2. Specific objectives	4
1.3. Significance of the study.....	4
2. LITERATURE REVIEW	5
2.1. Etiology.....	5
2.2. Types of mastitis	7
2.2.1. Clinical mastitis	7
2.2.2. Subclinical mastitis	7
2.3. Epidemiology.....	8
2.4. Transmission.....	9
2.5. Risk factors	10
2.5.1. The Host factors.....	10
2.5.2. The pathogenic factors.....	11
2.5.3. The environmental factors	11
2.6. Impacts of bovine mastitis	12
2.6.1. Economic impacts.....	12
2.6.2. Impacts on public health	13
2.7. Diagnosis	14
2.7.1. Clinical diagnosis.....	14

2.7.2. California mastitis test	14
2.7.3. Somatic cell count.....	15
2.7.4. Bacteriological tests.....	15
2.7.5. Biochemical tests	16
2.7.6. Molecular diagnostic techniques	17
2.8. Treatment	18
2.9. Prevention and Control	19
2.10. Prevalence of bovine mastitis in Ethiopia.....	20
3. MATERIALS AND METHODS	22
3.1. Study area	22
3.2. Study animals.....	23
3.3. Study design.....	24
3.4. Sampling techniques and sample size determination	24
3.5. Study methodology.....	26
3.5.1. Clinical examination of lactating cows.....	26
3.5.2. California mastitis test	26
3.5.3. Milk sample collection.....	27
3.5.4. Bacteriological and biochemical tests.....	27
3.6. Questionnaire survey	28
3.7. Data management and analysis.....	28
4. RESULTS	30
4.1. Prevalence of mastitis	30
4.2. Risk factors	32
4.3. Bacterial isolation	36
5. DISCUSSION	37
6. CONCLUSION AND RECOMMENDATIONS.....	43
REFERENCES.....	44
ANNEXES	59

ACKNOWLEDGMENTS

First of all, I would like to thank Almighty God, for His support throughout my life and allowing me to arrive at this moment and position. Next, I would like to thank my advisors, Dr. Gizachew Hailegebreal and Dr. Mesele Abera, for their guidance, provision of valuable advice, and devotion of time throughout writing this MSc thesis starting from title selection. Also, I would like to thank Dr. Amene Fikadu, Dean of the Faculty of Veterinary Medicine for providing CMT reagent and other laboratory facilities. I would like to acknowledge Hawassa University Veterinary Microbiology Laboratory for giving me the opportunity to carry out my laboratory work. I would also like to acknowledge the Dara district Livestock Resource Development Office staff for their cooperation during data collection. Finally, I would like to thank my family and all my friends who have guided and supported me directly and indirectly in the preparation of this MSc thesis.

DEDICATION

I dedicate this thesis manuscript to my father, Shabula Binbo, and my mother, Worke Orisa, for nursing me with affection and love and for their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

I declare that this thesis is my original work, and all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Zerihun Shabula (zerihunshabula02@gmail.com)

Signature: _____

Place: Faculty of Veterinary Medicine, Hawassa University, Hawassa

Date of Submission: _____

LIST OF ABBREVIATIONS

CAMP	Christie Atkins Munch-Peterson
CI	Confidence Interval
CMT	California Mastitis Test
CSA	Central Statistical Agency
DCT	Dry Cow Therapy
DNA	Deoxyribose Nucleic Acid
DDFEDO	Dara District Finance and Economy Development Office
DDLRO	Dara District Livestock Resource Development Office
FAO	Food and Agricultural Organization
IMViC	Indole Methyl red Voges-Proskauer Citrate
masl	Meter Above Sea Level
NAAS	National Academy of Agricultural Science
NMC	National Mastitis Council
PCR	Polymerase Chain Reaction
SCC	Somatic Cell Count

LIST OF TABLES

	Page
Table 1: Prevalence of bovine mastitis in different parts of Ethiopia	21
Table 2: Proportionally allocated number of study animals within selected kebeles	25
Table 3: Prevalence of mastitis at the cow and quarter-level	30
Table 4: Prevalence of mastitis at the quarter-level.....	30
Table 5: Prevalence of mastitis by kebele	31
Table 6: Univariable logistic regression analysis for the association of different risk factors with the occurrence of mastitis	34
Table 7: Multivariable logistic regression analysis for the association of different risk factors with the occurrence of mastitis	35

LIST OF FIGURES

	Page
Figure 1: Epidemiologic disease triad	9
Figure 2: Map of the study area	23
Figure 3: Bacterial isolates from milk samples	36

LIST OF ANNEXES

	Page
Annex I: Questionnaire survey format.....	59
Annex II: Animal data collection format during milk sample collection.....	62
Annex III: Figures for CMT test and laboratory examination of milk sample.....	63

ABSTRACT

Mastitis is one of the most significant diseases of dairy cattle worldwide. It causes major economic losses as it adversely affects animal health, degrades milk quality and quantity and impairs public health. A cross-sectional study was conducted from October 2022 to June 2023 to estimate the prevalence of mastitis, assess the associated risk factors and isolate major bacterial pathogens in lactating cows in Dara district, Sidama-Ethiopia. A total of 380 lactating cows were examined to detect mastitis using clinical examination, CMT test and bacteriological methods. Stata software version-14 was used to perform statistical analysis. An overall prevalence of mastitis in this study was 24.2% (95% CI: 20.1-28.8) with 21% subclinical and 3.2% clinical mastitis at cow level and 11.3% (95% CI: 9.4-13.4) with 9.5% subclinical and 1.8% clinical mastitis at quarter level. From observed risk factors breed, parity, management system, dry cow therapy, washing udder and milking mastitic cow last showed significant association ($p < 0.05$) with the occurrence of mastitis in the multivariable logistic regression model. From milk samples cultured for bacteriological examination, *S. aureus* (32, 40.5%) followed by *Streptococcus* species (20, 25.3%), Coagulase negative *staphylococci* (133, 16.5%), *E. coli* (11, 13.9%) and *Klebsiella pneumoniae* (3, 3.8%) were found to be the dominant bacterial isolates. The study revealed that mastitis is a significant problem for dairy cows, public health, and economy in the study area. Based on the study findings, improving the awareness of dairy farmers on the impact of mastitis, developing the hygienic status of cows and materials, and using of dry cow therapy are recommended as possible solutions for the prevention and control of mastitis.

Key words: *Bacteria, Dara, Lactating Cows, Mastitis, Prevalence, Risk Factors*

1. INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. In the country dairy cows are estimated to be about 12.57 million, and of these, 7.15 million are milking cows (CSA, 2020). In Ethiopia dairy cattle are primarily kept for milk production and attention is given to improving their milk production capabilities (Lijalem *et al.*, 2015). Development of the dairy sector can contribute immensely to income generation, employment opportunities, poverty alleviation and improved nutrition in the country. However dairy production in Ethiopia is constrained by several factors that cause a decline in milk production. A few of them are feed and water shortages, the poor genetic makeup of breeds, and the incidence of animal diseases (Guadu and Abebaw, 2016). Diseases pose a significant threat to dairy cattle production. Among the major diseases affecting dairy cattle, mastitis is reported to be the most severe disease of high prevalence in dairy farms resulting in production and economic losses (Sayeed *et al.*, 2020).

Mastitis is an inflammation of the mammary gland and the most common disease of dairy cattle which can reduce milk yield and alter milk composition. It causes enormous financial losses as it adversely affects animal health and the quality and quantity of milk (Shiferaw and Telila, 2016). Mastitis is a multi-etiological disease, and many microorganisms implicated as causative agents of mastitis are bacteria, fungi and algae. Major bacteria that most frequently cause mastitis in dairy cows are *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *Escherichia coli*, and other coliform bacteria (Cervinkova *et al.*, 2013).

Mastitis can be classified as clinical or subclinical forms based on the presence or absence of observable manifestations of clinical signs. Clinical mastitis is characterized by sudden onset, the presence of one or more clinical signs like udder swelling and change in milk color, and systemic signs, such as elevated body temperature, anorexia, depression, increased heart rate and lethargy (Pandey *et al.*, 2012). Subclinical mastitis is the most common form and is considered to be the most economically important due to its higher prevalence, difficulty in recognition, and catastrophic long-term effects compared to clinical mastitis (Elango *et al.*, 2010).

Several numbers of predisposing factors are implicated as the causes of bovine mastitis including breed, age, parity, stage of lactation, udder hygiene, animal husbandry practices, milker's hand, quality of water, incomplete milking, sanitation in the animal shed, and awareness of dairy farmers (Pal, 2018). Diagnosis of clinical mastitis is less complex, because clinically discernable signs, including swollen udder and teat and poor milk quality, can be detected by farmers (Mahmmod, 2013). By contrast, subclinical mastitis cannot be visually diagnosed and it requires the application of diagnostic techniques. Various methodologies are suggested for the diagnosis of mastitis, and some of these are clinical examination, California mastitis test (CMT), somatic cell count (SCC) and bacteriological examination (Lam *et al.*, 2009).

Control of mastitis requires an understanding of its causes and management techniques which limit the spread of infection. Management of udder health during the dry season is critical for a smooth transition into the lactation period (Ondiek *et al.*, 2013). The key elements that need

to be considered for the control of mastitis include sound husbandry practices and sanitation, post-milking teat dipping, treatment of mastitis during the non-lactating period, and culling of chronically infected animals.

1.1. Statement of the problem

Bovine mastitis is one of the most costly, devastating diseases in the dairy industry. Both subclinical and clinical features of the disease cause substantial financial losses due to reduced milk yield, degraded milk quality, drug costs and veterinary expenses, early culling of dairy cows, and increased labor costs for the farmers (Abdeta and Gemechisa, 2020). A disease has been reported in different parts of Ethiopia, with an overall prevalence of 40.3% (Tezera and Aman, 2021); 73% (Etifu and Tilahun, 2019); 24.5 % (Yohannes and Alemu, 2018); 64.3% (Zerihun and Abera, 2017); 42.7% (Tekle and Berihe, 2016); 68.0% (Tilahun and Aylate, 2015) and 37.1% (Abera *et al.*, 2012). Dara district has a high cattle population and is working efficiently to improve the genetic makeup of the breed for high milk production. Also there are organized farmers cooperatives in the area that collect milk from several farmers and supply it to consumers. Milk collected from several farmers mixed before reaching the consumer, and as a result pathogens from mastitic milk have the chance to enter into healthy milk causing human health hazards. However, there is no information about the status of mastitis in lactating cows in this area for designing prevention and control strategies, so the prevalence of bovine mastitis should be known in this area.

1.2. Objectives of the study

1.2.1. General objective

To estimate the prevalence of mastitis, its associated risk factors and to isolate and identify major bacterial causes of mastitis in lactating cows in Dara district, Sidama-Ethiopia.

1.2.2. Specific objectives

- ❖ To estimate the prevalence of mastitis in lactating cows in the study area.
- ❖ To isolate and identify major bacterial causes of mastitis.
- ❖ To assess the potential risk factors associated with the prevalence of mastitis.

1.3. Significance of the study

Mastitis is one of the major diseases of dairy cows in Ethiopia. Therefore, the result of this study helps stakeholders to design and implement strategies for prevention and control of mastitis in the study area. Also, farmers will be aware of the impacts of mastitis, especially subclinical mastitis, and improve the ways of prevention and control of the distribution of the mastitis. In addition, this study could be used as baseline information for further studies in the area.

2. LITERATURE REVIEW

2.1. Etiology

Nowadays, there are more than 140 potentially pathogenic species that cause bovine mastitis. Based on the pathogen involvement, mastitis-causing microorganisms are broadly classified as bacteria, fungi and algae. Viruses have the least clinical significance (Bachaya *et al.*, 2011). Major bacterial species that are responsible for the causes of mastitis could be *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. bovis*, coliform bacteria (e.g., *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* species), *Corynebacterium bovis*, *Mycoplasma* species, *Serratia*, *Pseudomonas*, *Proteus* species etc. (Girma *et al.*, 2012).

The genus *Staphylococcus* is a gram-positive pathogen known to be the most common mastitis-causing in ruminants in many countries and based on the coagulase test genus *Staphylococcus* is classified as coagulase-positive and coagulase-negative species (Abebe and Bakala, 2022). *Staphylococcus aureus* is a major mastitis-causing species, producing both subclinical and clinical infections. The incidence of *S. aureus* infections has been found to be higher during early lactation (Saidi *et al.*, 2013). One characteristic of *S. aureus* is the ability to produce a biofilm during pathogenesis, which protects the pathogen from phagocytosis by neutrophils and macrophages (Olde *et al.*, 2008)

Genus *Streptococcus* belongs to the gram-positive group of bacteria, round-shaped with chain formation. Mostly, *Streptococcus* is a common bacteria found in the environment, humans, and animals. *Streptococcus* species are often found in mastitis, include *S. agalactiae*, *S.*

dysgalactiae, and *S. uberis* (Abebe and Bakala, 2022). *Streptococcus agalactiae* is classified as a contagious pathogen. *Streptococcus dysgalactiae* is able to survive within the host and in the environment; this uncertainty makes this species counted as an intermediate pathogen. *Streptococcus uberis* is mainly classified as an environmental pathogen, nonetheless contagious case has also been studied (Sumathi *et al.*, 2008). *Streptococci* possess different virulence factors. Biofilm is one of the virulence factors which allow this species to survive in the bovine udder and cause subclinical mastitis (Cheng and Han, 2020).

Escherichia coli and *Klebsiella* are coliform bacteria that can cause bovine mastitis. These bacteria are part of the normal bovine intestinal flora and contaminate the environment, often bedding material, via feces (Sadek *et al.*, 2017). Among coliforms bacteria, *E. coli* is the most frequently isolated from bovine milk in cows belonging to dairy farms with intensive systems of milk production. *E. coli* is a member of the family Enterobacteriaceae and most frequently induces acute clinical mastitis, often of serious character with rapid progress and sometimes with a fatal outcome (Etifu and Tilahun, 2019; Seid *et al.*, 2015; Mekibib *et al.*, 2010). *E. coli* can also cause subclinical mastitis, although less frequently. *Klebsiella* spp. in general, causes severe but less rapidly developing clinical mastitis cases than *E. coli* but with a longer duration of infection and may give rise to subclinical mastitis (Getahun *et al.*, 2008).

2.2. Types of mastitis

2.2.1. Clinical mastitis

Clinical mastitis refers to the condition where the cow's immune system responds to etiological agents and is characterized by signs of inflammation in the mammary gland including hyperemia, pain, increased gland size and density, and discoloration of milk (Mahantesh *et al.*, 2011). These signs may or may not be accompanied by systemic signs, such as fever or depression. Clinical mastitis can be further subdivided into per-acute, acute, subacute, and chronic, depending on the degree of the inflammation (Lakew *et al.*, 2009). Per-acute mastitis has an extremely severe and rapid onset, which frequently results in the animal's death. The onset of an acute clinical infection is relatively quick, and signs include the mammary gland appearing red, swollen udder, and changes in milk consistency. Cows with acute clinical mastitis also experience systemic signs (Kemp *et al.*, 2008). Both acute and per-acute forms of clinical mastitis are hazardous for the cow as infection affects the animal systemically. Subacute mastitis is the most common type of clinical mastitis, which has a slight inflammation and swelling to the mammary gland accompanied by a change in milk consistency. Chronic cases are the least severe compared to the other types of clinical mastitis, as there is a visible change in milk consistency and infection (Roberson, 2012). The most important bacteria that can cause clinical mastitis are *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *S. uberis*, *E. coli*, and *Klebsiella* spp. (Tekle and Berihe, 2016).

2.2.2. Subclinical mastitis

Subclinical mastitis is considered to be a more important form of mastitis due to its higher prevalence, difficulty in recognition, and catastrophic long-term effects compared to clinical

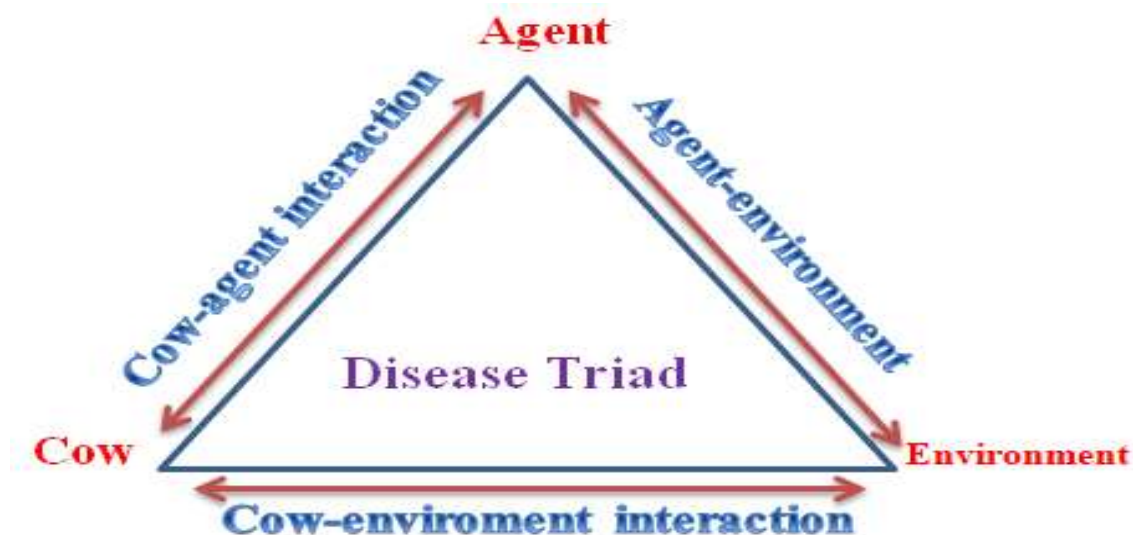
mastitis (Pandey *et al.*, 2012). In addition, subclinical mastitic cows considered a microbial reservoir in the herd, increase the exposure of healthy cows to clinical mastitis (Kumar *et al.*, 2009).

In subclinical mastitis, there are no visible abnormalities in udder tissues and physical appearance of the milk, except an elevated number of SCC, reduction of milk production, presence of bacteria in the milk and altered milk composition (Atasever and Erdem, 2009). Variations in milk composition, such as somatic cell count, leukocytes, and epithelial cells, as well as changes in milk pH and ion concentration, are all indicators of subclinical mastitis. Subclinical mastitis is defined by quantification of somatic cell count in milk. In dairy cows, a somatic cell count higher than 200,000cells/ml defines the diagnosis of subclinical mastitis (Olivares-Pérez *et al.*, 2015).

2.3. Epidemiology

There are two distinct patterns of infection in the epidemiology of mastitis that can be recognized as contagious and environmental/opportunistic infectious patterns. Contagious disease pattern is the most common mode where transfer of a microorganism from cow to cow is essential to propagate the disease. This pattern involves the transmission of disease from a carrier to a susceptible host (Shiferaw and Telila, 2016). An opportunistic disease pattern is the second pattern of disease where the infection occurs mainly in housed or closely corralled cattle. The causation of mastitis involves the interaction of three major complex factors. These are microorganism (agent), host animals and environment (figure 1). Host factors include breed, physiological state of the mammary gland, and anatomy of the teat canal, sphincter tone

and presence of teat lesion. Agent factor includes the ability to survive in the immediate environment of the animal, the ability to colonize the teat duct, and the ability to adhere to the mammary epithelium and not to be flushed out with milk flow. Environmental factor includes milking practice, housing system and bedding (Ismael, 2018).



Source: Thrusfield *et al.* (2018)

Figure 1: Epidemiologic disease triad

2.4. Transmission

Based on the mode of transmission, mastitis-causing pathogens can be classified as contagious and environmental pathogens. Contagious mastitis is caused by pathogens that live and multiply on and in the cow's mammary gland. These pathogens may spread from cow to cow and are the primary source of infection (Zadoks *et al.*, 2011). Major pathogens that cause contagious mastitis include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp., and *Corynebacterium bovis*. These contagious pathogens reside mainly in the udder of infected cows and are spread by contaminated milking equipment, milker's hands, or any

other materials that act as carriers (Katsande *et al.*, 2013). Incidence of contagious mastitis depends on the dose and type of microbes to which a cow is exposed as well as physical barriers and the innate and acquired defense mechanisms (Halasa *et al.*, 2009).

Environmental mastitis is caused by organisms that do not usually live on the surface of the skin or in the udder but which enter the teat canal when the cow comes into contact with a contaminated environment. The primary source of environmental pathogens is the surroundings in which a cow lives (Bytyqi *et al.*, 2010). Those pathogens causing environmental mastitis include *Streptococcus uberis*, *Streptococcus dysagalactiae*, coliform bacteria, etc. They reside in the environment (bedding, flooring, droppings) and are transmitted at any time of a cow's life: during milking, between milking, during the dry period, and during parturition (Radostits *et al.*, 2007).

2.5. Risk factors

2.5.1. The Host factors

The host factors that can influence the causes of mastitis include age, breed, parity, stage of lactation, udder and teat morphology and level of milk production (Huijps *et al.*, 2008). Older cows are more susceptible to infections than adults, most probably because of the permanently partially open teat canal as a result of frequent milking. Furthermore, the mammary epithelium of older cows has increased permeability, mainly because of the irreversible damage caused by previous inflammations (Król *et al.*, 2013). The highest prevalence is in the pure Holstein breeds, followed by Jersey, cross breed and indigenous zebu being less frequently affected than others. This might be due to differences in the anatomical structure of the teats and

differences in the capacity to resist the disease (Persson *et al.*, 2009). According to Yusuf *et al.* (2023) and Moges *et al.* (2012), the prevalence of mastitis is higher in cows with higher milk production than cows with low milk yields and also highest in cows with early lactation stage compared to cows at mid and late lactation stages.

2.5.2. The pathogenic factors

Many bacterial agents of mastitis have virulence factors like adherence components for attachment, ability to colonize, capsule formation, presence of virulence genes on large plasmids, production of toxins, bacterial viability, biofilm formation, and resistance to antibiotics (Radostits *et al.*, 2007). For example, *E. coli* produces lipopolysaccharide endotoxin, which is responsible for many of the inflammatory changes observed during coliform mastitis; and *S. aureus* isolated from intramammary infections produce many potential virulence factors, including enterotoxins, hemolysin, hyaluronidase, and leukocidins. Some mastitis pathogens also show resistance to the commonly used antibiotics. For instance, some *Staphylococci* produce the β -lactamase enzyme, which makes these bacteria resistant to penicillin (Adane *et al.*, 2012).

2.5.3. The environmental factors

The design of the housing system, hygiene, and herd size of dairy cows, milking practice, and climatic conditions interact to influence the degree of exposure of a cow to mastitis pathogens (Abunna *et al.*, 2013). Moisture, mud and manure present in the environment of the animals are primary sources of exposure to environmental mastitis pathogens (Mekonnen *et al.*, 2016). Moreover, the occurrence of mastitis varies from season to season, because growth and

multiplication of organisms depend on specific temperature and humidity. Incorrect ventilation with high temperatures and relative humidity encourages the multiplication of various bacteria. Exposure of animals to high temperatures can increase the stress of the animal and alter immune functions (NAAS, 2013).

2.6. Impacts of bovine mastitis

2.6.1. Economic impacts

Bovine mastitis is considered to be one of the most economically significant diseases of the dairy industry in the world (Singh *et al.*, 2014). Following the occurrence of the disease, economic costs due to mastitis are expressed in two terms: losses and expenditures. The economic losses imply the losses of a benefit that is taken away in case of mastitis, or it represents a potential benefit that is not realized, or it is reduced revenue. For example, the production loss experienced due to a decrease in the milk yield or discarded milk because of contamination. Some studies showed that average annual milk losses due to mastitis are 22.3% in cross-breed and 2.24% in local zebu cattle (Beyene and Tolosa, 2017). The three main components of the economic impact due to mastitis were the culling of cows, reduction in milk production, and costs for disease control, which account for 39.4%, 32.3% and 18.2%, respectively (Juliana *et al.*, 2017). According to FAO (2014), in Ethiopia, a total loss of US\$ 38 per cow per lactation was estimated. In contrast, expenditures represent some economic effects of a disease that are manifested as extra inputs into livestock production or extra resources used, such as treatment and prevention of mastitis (Olivares-Pérez *et al.*, 2015).

In general, major economic costs associated with bovine mastitis are losses of milk production, drug costs, losses due to discarded milk, cost of veterinary services, extraneous labor costs, losses due to changes in product quality, costs of materials and investments, diagnostics cost, losses due to mortality and culling, costs of cow replacement (Radostits *et al.*, 2007). Moreover, some reports indicate that the mortality rate due to mastitis is 0.6 %, in general and 2.2 % due to Gram-negative organisms (Sharif and Muhammed, 2009).

2.6.2. Impacts on public health

The transmission of several zoonotic diseases can occur in a variety of ways, including through the ingestion of tainted milk and close contact with infected animals and animal products. Milk is a well-known vehicle for a number of human pathogenic microorganisms (Shiferaw, 2009). Milk from mastitic cows carries bacteria that can cause human illness when raw milk is consumed or when pasteurization is faulty. The most crucial human disease-causing bacteria that can be found in milk are *Mycobacterium bovis* and *M. tuberculosis*, *Brucella species*, *Salmonella species*, *E. coli*, *Staphylococcus aureus*, *Streptococcus pyrogen*, and *Corynebacterium haemolyticum* (Lakew *et al.*, 2009).

Mycobacterium bovis can spread to humans through the consumption of raw milk or unpasteurized or improperly pasteurized milk and dairy products from infected animals. According to Hassanain *et al.*, (2009), 4.35% of *M. tuberculosis* is transmitted through contaminated milk. Brucellosis is a zoonotic disease that can be transmitted to humans predominantly by the consumption of contaminated raw milk and dairy products. High contamination rates (72.5%) were found in raw milk (Abdali *et al.*, 2020). Some strains of

Staphylococcus aureus may produce heat resistance enterotoxins and toxic shock syndrome toxin-1 (TSST-1), which cause severe food poisoning (Cheng *et al.*, 2010). *Streptococcal* species cause clinical and subclinical mastitis in animals and scarlet fever, sore throats, tonsillitis, bacterial endocarditis, rheumatic fever, and pneumonia in humans (Manning *et al.*, 2010). Another public health concern regarding mastitis is antibiotic residues in milk, which can initiate severe allergic reactions in humans to antibiotics and at low levels can cause sensitization of normal individuals and the development of antibiotic resistance strains of bacteria (Galal *et al.*, 2007).

2.7. Diagnosis

2.7.1. Clinical diagnosis

Clinical examination is performed through visual examination of the milk and udder, and by palpation of the mammary gland. Clinical examination aims is to detect swelling, pain reaction, symmetry, consistency, the blindness of the udder and teats, and examination of milk for discolorations, blood ting, wateriness, clots, flakes, and pus (Quinn *et al.*, 2004). Clinical mastitis may recognized by signs of inflammation like swelling, pain, redness, and heat in case of acute mastitis. Whereas, hardening of the udder, blockage of the teats, atrophy or fibrosis, and abscess formation are manifested in chronic mastitis (Moges *et al.*, 2012).

2.7.2. California mastitis test

California mastitis test is a simple and rapid screening test for mastitis. It is valuable technique for detecting subclinical mastitis on a farm, providing an immediate result, and for selecting the samples for bacterial culturing from the cows under study (Quinn *et al.*, 2004). It is carried

out by collecting a squirt of milk sample from each quarter onto shallow cups of CMT paddle and mixing with an equal amount of CMT reagent. The reaction is interpreted based on the thickness of the gel formed (Qolbaini *et al.*, 2014). The degree of reaction between a reagent and the DNA of cell nuclei indicates the concentration of somatic cells in a milk sample; however, it does not identify the type of bacteria that cause mastitis (Tolosa *et al.*, 2013).

2.7.3. Somatic cell count

The somatic cells are the normal constituent of milk that have been shed from the lining of the mammary gland and consist of white blood cells and epithelial cells (Bytyqi *et al.*, 2010). Somatic cell counts in milk from healthy mammary glands usually contain about 100,000 cells/ml. In contrast, the number of SCC above 200,000 cells/ml is an indicator of an immune response to the presence of infection in the mammary gland (Durr *et al.*, 2008). The contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*) generally cause the most significant SCC increase. An infection by environmental pathogens (*Streptococcus dysgalactiae*, *Streptococcus uberis*, *Corynebacterium bovis*, and coagulase-negative *Staphylococcus*) usually causes considerably less SCC elevation (Sharma *et al.*, 2011). Somatic cell counts are widely used to predict the mammary gland health status of cows and quarters, the suitability of milk for human consumption, and monetary losses to producers due to mastitis (Petzer *et al.*, 2016).

2.7.4. Bacteriological tests

Bacteriological culturing is most often used as a diagnostic tool to solve mastitis problems. Knowledge on the infectious status of mammary glands, however, can also be beneficial to

prevent the transmission of pathogens by diagnosing a reservoir at an early stage. To effectively use bacteriological culturing as a diagnostic tool, milk samples have to be collected from the correct cows and quarters at the correct point in time (Sharif and Muhammed, 2009). Proper collection of milk samples is of paramount importance for the identification of mastitis pathogens. The aseptic technique is an absolute necessity when collecting milk samples to prevent contamination by organisms found on the cows' skin, udder, and teats, the hands of the sampler; and in the barn environment. Contaminated samples result in misdiagnosis, increased work and expense, confusion, and frustration (Kumar *et al.*, 2009).

Bacteriological culturing can be executed at the herd, as well as cow and quarter level, each with its own specific goal. Culturing of milk samples for bacterial isolation is very important to detect the antimicrobial susceptibility of bacteria and to provide optimal treatment protocols and control measures for cows with clinical and subclinical mastitis. Bulk tank milk can also be cultured to diagnose the presence and prevalence of mastitis pathogens on a herd basis. Also milk from individual cows can be cultured as part of a herd examination for mastitis or on individual quarter samples (Ararsa *et al.*, 2014).

2.7.5. Biochemical tests

Biochemical tests are used for the identification of bacteria based on differences in the biochemical activities among them (Muhammad *et al.*, 2013). Such as the metabolism of carbohydrates, protein, and fat; and production of certain enzymes, like coagulase, Nacetyl- β -D-glutaminidase (NAGase), urease, catalase, etc., and the ability to utilize a particular compound (Ravea *et al.*, 2019). There are many biochemical tests available. The primary

biochemical tests are the catalase test and oxidase tests, and secondary biochemical tests include the Indole test, Methyl red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test, Triple sugar iron test, and coagulase test. Biochemical tests used for the identification of *Staphylococcus* are the catalase test, coagulase test, Vogas Proskauer (VP), urease test, and thermonuclear test; for the identification of *Streptococcus* species are sugar fermentation, catalase tests, asculin hydrolysis, etc. and for the identification of *E. coli* IMViC tests are used (Quinn *et al.*, 2004).

2.7.6. Molecular diagnostic techniques

Molecular diagnostic techniques are used for bacterial identification. When compared to culture-based approaches, molecular-based techniques have been proven to be more sensitive and more rapid since their findings may be ready in only a few hours (Adkins and Middleton, 2018). Genotypic methods use DNA as the basis for the identification of species and strain type. PCR is used to improve the diagnosis of pathogens for which biochemical methods for the identification of species are known to be inaccurate (Mahmmod, 2013). It is noted that PCR testing is helpful to detect the presence of obligate udder pathogens like *S agalactiae* in bulk milk, especially when there is a low prevalence of infected cows within the herd (Giuliano *et al.*, 2019). The high sensitivity of PCR, which is capable of detecting a single molecule of DNA, may be seen as an advantage for microbiological diagnostic purposes.

Up to 30% of clinical mastitis samples yield no growth in bacterial culture, but PCR analysis is sensitive enough to detect growth-inhibited and non-viable bacteria (Taponen *et al.*, 2009). This leads to a possible decrease in the rate of false-negative results. In addition, short

throughput times and the potential for objective and user-independent identification are other arguments in support of PCR assays. Identification of non-viable bacteria has the potential to enable the integration of intramammary infection diagnostics (Tesfaye, 2016).

2.8. Treatment

Based on the stages in the lactation cycle, the treatment of bovine mastitis can be classified as lactating cow therapy and dry cow therapy. Dry cow therapy (DCT) is one of the best choices to control and inhibit progression of mastitis. The dry period is a crucial stage in the lactation cycle; any infection during the dry period will affect the next lactation, and therefore, it is essential to take care of the cow's health before the next lactation cycle. Before drying off the cows, they have to be checked for any sign of mastitis; chronic mastitis cases, which are difficult to detect by the naked eye, should be checked via the California mastitis test (Hossain *et al.*, 2017).

Dry cow therapy is an antibiotic treatment administered to cows during a dry period. The benefits of DCT are related to decreasing the number of infectious organisms within the mammary gland, thus reducing the incidence of new intramammary infection post-partum (Basappa *et al.*, 2011). In comparison to treating mastitis infections during lactation, the dosage of antibiotics is less compared to DCT due to the risk of antibiotic residues within the milk. Therefore, cows receiving DCT a dry period is more beneficial since higher dosages can be administered and the incidence of new intramammary infection can be decreased (Jones, 2009). The successful therapeutic regimens of bovine mastitis depend on the etiology, clinical presentation, and antimicrobial susceptibility of the etiological agent. The most commonly

available antimicrobial drugs used for the treatment of bovine mastitis are penicillin, aminoglycosides, tetracycline, sulphonamides and trimethoprim, macrolides, enrofloxacin, cephalosporin and polymyxins (Verma *et al.*, 2018).

2.9. Prevention and Control

Complete eradication of mastitis is not possible, as it is a disease caused by multiple etiologies and complex factors. However, the control of mastitis can be successfully achieved through the establishment of effective herd health control programs. Early diagnosis of mastitis with reliable tests can facilitate successful treatment and control (Sharif and Muhammed, 2009). The fundamental principle of mastitis control is controlling the disease by either decreasing the exposure of the teat to potential pathogens or by increasing the resistance of dairy animals to infection (Islam *et al.*, 2010). Control of contagious mastitis pathogens is focused on reducing exposure of teats to pathogens that originated from infected cows. Control of environmental mastitis can be achieved by reducing the number of bacteria found around cows' environment, increasing the immune resistance of the cows, pre-milking teat dipping with a germicidal (Radostits *et al.*, 2007).

The key elements that need to be considered for the prevention and control of bovine mastitis are employing proper milking management methods and proper use of milking equipment, such as washing hands with soap and water, washing teats and udder in sanitizing solution, thoroughly drying teats and udder with individual towels, dipping teats in an effective germicidal teat dip, thoroughly scrubbing the teat end with a cotton swab soaked in alcohol (DeVliegher *et al.*, 2012). Dry cow management, appropriate therapy during lactation, culling

chronically infected cows, maintenance of a clean environment, monitoring udder health status, periodic review of the udder health program, good record keeping, and setting goals for udder health status are some important points to control. The use of dry cow therapy can help eliminate existing infections and prevent new infections during the early dry period (Fadlelmula *et al.*, 2009).

2.10. Prevalence of bovine mastitis in Ethiopia

Over the last several years, a number of studies have been available that describe the prevalence of bovine mastitis in different parts of Ethiopia (Matios *et al.*, 2009; Zerihun *et al.*, 2013; Abebe *et al.*, 2016; Mekibib *et al.*, 2010). Also a number of epidemiological studies carried out in Ethiopia showed that, mastitis is a severe problem in the country. In Ethiopia, the prevalence reports were heterogeneous and were affected by several factors (Tesfaye, 2016; Kebebew and Jorga, 2016). The variability in the prevalence of mastitis among different studies conducted in different areas of the country might be attributed to differences in management practices, environmental conditions in the study areas, breed of animals, diagnostic methods used, and other factors. In Ethiopia, studies on the prevalence of bovine mastitis were usually made through clinical examination of the udder in cases of clinical mastitis and through the use of one of the cow side tests, especially CMT, followed by indicator paper test for subclinical cases (Seid *et al.*, 2015; G/Michael *et al.*, 2013; Girma *et al.*, 2012). Also, bacteriological methods are used for clinical and subclinical cases for isolation and identification of the causative agents (Zenebe *et al.*, 2014; Birhanu *et al.*, 2017; Abera *et al.*, 2013; Haftu *et al.*, 2012). Moreover, in Ethiopia, different authors have reported varying ranges of clinical and subclinical cases of mastitis in different areas (Table 1).

Table 1: Prevalence of bovine mastitis in different parts of Ethiopia

Study area	Region	No. of animal tested	No. of positive animals	Prevalence (%)	Subclinical mastitis (%)	Clinical mastitis (%)	References
Adigrat	Tigray	322	207	64.3	54.7	9.6	Zenebe <i>et al.</i> (2014)
Ambo town	Oromia	384	161	42	32.6	9.4%	Ejeta <i>et al.</i> (2022)
Asossa town	Benshangul	367	148	40.3	28.34	11.99%	Tezera and Aman (2021)
Bishoftu	Oromia	262	105	40.1	40.1	-	Birhanu <i>et al.</i> (2017)
Doba, West Hararghe	Oromia	384	89	23.18	15.89		Girma <i>et al.</i> (2012)
Gamo zone	SNNP	422	72	17.1	1.9	15.2%	Belay <i>et al.</i> (2022)
Hawassa	SNNP	529	331	62.6	59.2	3.4	Abebe <i>et al.</i> (2016)
Holleta town	Oromia	107	76	71	48.6	22.4	Mekibeb <i>et al.</i> (2010)
Jikawo Woreda	Gambella	121	73	60.33	48.76	11.57	Deng <i>et al.</i> , (2015)
Kombolcha	Amhara	150	84	56	46	10	Tesfaye (2016)
LemuBilbilo, Arsi Zone	Oromia	300	126	42	36.7	5.3	Biressa and Tesfaye (2015)
Haramaya	Harari	56	24	42.9	30.4	12.5	Yusuf and Husen (2023)
Modjo town	Oromia	384	283	73.7	52.3	21.4	Fesseha <i>et al.</i> (2022)
Shashemene town	Oromia	245	91	37.1	28.6	8.5	Abera <i>et al.</i> (2012)
Sidama Zone	SNNP	96	43	44.78	42.7	2.08	Tekle and Berihe (2016)
Wolayta Sodo	SNNP	245	60	24.5	4	19.6	Yohannes and Alemu (2018)
Bahir Dar	Amhara	302	85	28.2	25.2	3	Bitew <i>et al.</i> (2010)
Yabello	Oromia	460	272	59.1	21.1	38	Adane <i>et al.</i> , (2012)

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted from October 2022 to June 2023 in and around Kebado town, Dara district Sidama-Regional State. Dara district is one of the districts found in Sidama-Regional State. The district is situated 85km from Hawassa and 345km from Addis Ababa, the capital cities of Sidama Regional-State and Ethiopia, respectively. It is bordered on the north by the Dara-otilcho district, on the south by the Gedeo zone Dilla Zuria district and on the East by the Oromia region, Borena zone Abaya district, on the west by the Chuko district. The district is located at latitude 6°21'0"- 6°32'0" N and longitude 38°15'0"- 38°31'0"E. The altitude of the district ranges from 900m.a.s.l. to 2900m.a.s.l, and the mean annual rain fall ranges from 1201mm to 1700mm. The annual mean temperature of the district ranges from 12.6°C to 22°C and its total area is 15,292 hectares. Dara district is subdivided into 24 kebeles (peasant associations), and based on agro-ecological conditions it is also broadly categorized into three: 25.3% highland “Dega,” 54.4% midland “Woinadega” and 20.3% lowland “kola.” The total human population of the district is 141529, from which 69827 men and 71704 women (DDFEDO, 2021).

The total livestock population of the district is estimated to be 96,382 cattle, 74,104 sheep, 16474 goats, 2197 donkeys, 8422 horses, 728 mules, and 871306 poultry. The economic status of the people in the district is mostly dependent on a mixed farming system (crop and livestock production). In the district dairy farming system is commonly practiced by using local and improved (cross) breeds, where dairy production plays a crucial role in the

livelihood of the farming community. The management system of dairy cows is mainly extensive in rural areas, semi-intensive, and intensive in town. Moreover, traditional housing, feeding and milking procedures are mostly practiced (DDL RDO, 2022).

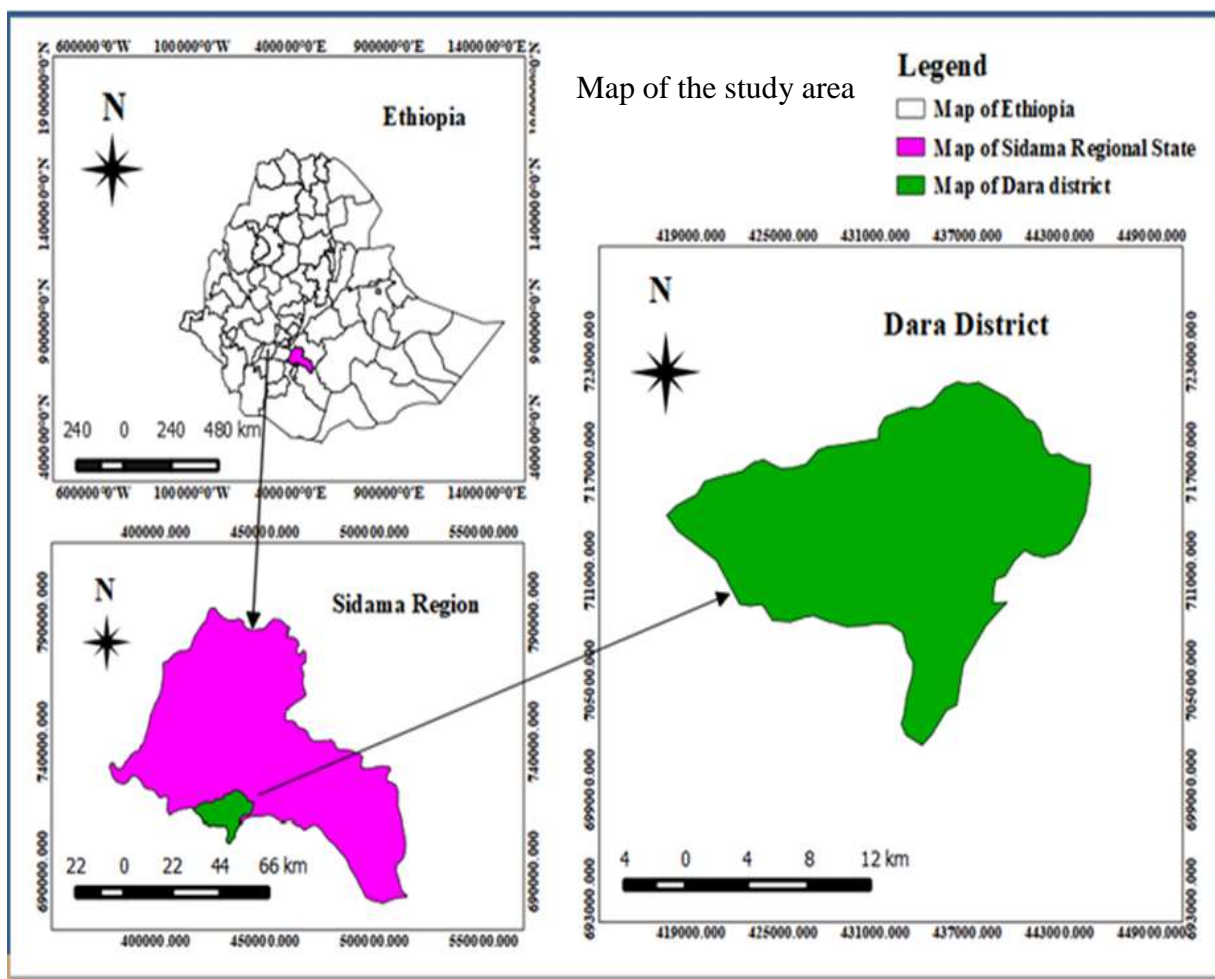


Figure 2: Map of the study area

3.2. Study animals

The total cattle population of a district is estimated to be 96,382 heads, and out of these, about 12,264 are dairy cows. Of the dairy cows referred to above, 8794 are lactating cows (DDL RDO, 2022). The study population was lactating cows found in the study area that are

kept under an intensive and extensive production system. The study animals consisted of 380 lactating cows which were selected by simple random sampling technique from the selected kebeles within a district. The breeds of study animals were categorized as zebu (local) and crossbred (zebu with Holstein-Friesian and zebu with Jersey). Age cows were grouped as young adult (3-5 years), adult (6-9 years), and old (>9 years). Parity was categorized as few (1-3 calves), several (4-6 calves), and many (>6 calves). The lactation stage of the cow was also categorized into early-lactation (1-4 months), mid-lactation (5-7 months) and late-lactation >7 months (Seid *et al.*, 2015).

3.3. Study design

A cross-sectional study was conducted from October 2022 to June 2023 to estimate the prevalence of mastitis, assess associated risk factors, and isolate major bacterial causes of mastitis in lactating cows in the study area.

3.4. Sampling techniques and sample size determination

In study areas, Kebado town and six rural kebeles were selected purposively based on the high number of dairy cows population according to data from the Dara District livestock resource development office (2022). Areas covered were Aleme-Korocho, Badessa, Gelo-Wacho, Kebado town, Korate, Machisho and Setamo. Herds and households were also selected purposively based on the presence of lactating cows. Finally individual lactating cows were selected by using a simple random sampling technique.

Table 2: Proportionally allocated number of study animals within selected kebeles

Selected Kebeles	Lactating Cows in the Kebeles (n)	Lactating cows sampled (n) (Calculated Sample Size)
Aleme-Korocho	571	62
Badessa	450	49
Gelo-Wacho	439	48
Kebado town	339	37
Korate	512	56
Machisho	484	53
Setsmo	683	75
Total	3478	380

The sample size required for this study was calculated according to the formula given by Thrusfield (2018), considering 44.78% prevalence based on a previous study undertaken in three districts (Aleta Wondo, HawellaTula, and Aleta Chuko) of Sidama-Ethiopia (Tekile and Berihe, 2016), 95% confidence interval and 5% desired absolute precision. Accordingly, the total number of samples required for this study was 380 lactating cows.

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where: n = required sample size (sample size of the study population),

P_{exp} = expected prevalence, and

d = desired absolute precision

3.5. Study methodology

3.5.1. Clinical examination of lactating cows

Clinical examination was performed on all selected 380 lactating cows and the cases were recorded. The manifestation of visible signs of inflammation of udder and abnormality of milk tentatively diagnosed clinical mastitis. Accordingly, swelling and warming of the udder and painful reaction upon palpation were considered acute clinical mastitis, whereas atrophied, hard, and fibrotic quarters were considered chronic mastitis (Quinn *et al.*, 2004; Radostitis *et al.*, 2007).

3.5.2. California mastitis test

California mastitis test was used to diagnose subclinical mastitis and also serves as the screening test for the selection of samples to be cultured for the cows under study. A squirt of milk sample from each quarter was collected into a plastic paddle that had four shallow cups marked A, B, C, and D. An equal amount of California mastitis test reagent was added to the milk sample. Then, the paddle was rotated gently to mix the contents. Positive samples showed gel formation within a few seconds. The CMT results were interpreted as negative (0), trace (T), weakly positive (+1), distinct positive (+2), and strongly positive (+3), as suggested by Quinn *et al.* (2004). All CMT scores of 0 and trace were considered negative. while CMT scores of +1, +2, and +3 were considered indicators of subclinical mastitis. Cows were considered positive for mastitis when at least one quarter was positive for CMT. A herd was considered positive for mastitis when at least one cow in the herd tested positive for CMT (Alemu *et al.*, 2013).

3.5.3. Milk sample collection

Milk samples were collected from each quarter of cows which are diagnosed with clinical or subclinical mastitis and screened positive for CMT according to the standard procedures recommended by (NMC, 2004). Approximately 10ml of milk sample was collected aseptically from lactating cows into sterile test tubes after discarding the first two squirts of milk from each quarter. After collection, samples were placed in an icebox and transported to Hawassa University Veterinary Microbiology Laboratory. Then, they were immediately cultured or kept there at 4°C until cultured on standard bacteriological media.

3.5.4. Bacteriological and biochemical tests

Isolation and identification of bacterial pathogens of mastitis were carried out at Hawassa University Veterinary Microbiology Laboratory on the samples diagnosed to have clinical mastitis and samples from CMT-positive cows for subclinical mastitis. The bacteriological culture was executed following the standard microbiological techniques recommended by Quinn *et al.* (2004) and (NMC, 2004). A loop full of milk samples was inoculated separately on to MacConkey agar and on a 5% sheep blood agar base. Then the plates were incubated aerobically at 37°C and examined after 24 hours of incubation for growth of bacteria. Primary identification of the bacteria was carried out on the basis of colony morphology, growth on media, hemolytic characteristics, pigmentation, catalase test and Gram staining. Colonies were also subcultures on the selective media such as Mannitol salt agar (MSA), MacConkey agar, and on the Blood agar plates for isolation of *Staphylococcus*, *Streptococcus*, and *E.coli* and *Klebsiella*, respectively. The identification and differentiation of bacterial isolates were performed through the following procedures.

Staphylococcus species were identified and differentiated by growth characteristics on mannitol salt agar, colony morphology, ability to produce hemolysis, catalase test, coagulase test, and Gram staining. *Streptococcus* was identified and differentiated by colony morphology, growth characteristic on media, ability to produce hemolysis (CAMP test), catalase test, and Gram staining. Coliform species (*E.coli* and *K. pneumoniae*) were also identified and differentiated by colony morphology on the MacConkey agar, Gram staining, motility test, triple sugar iron (TSI) agar and IMViC (Indole Methyl red Voges-Proskauer Citrate) Quinn *et al.* (2004).

3.6. Questionnaire survey

A semi-structured questionnaire format was used to collect cow-level and herd-level data along with collection of milk samples. Informants were owners/attendants of the cow from that milk sample had been taken. All data collected included age, breed, parity, stage of lactation, herd size, management system, dry cow therapy, udder washing practice before and after milking of individual cow, floor type, previous history of mastitis, milking mastitic cow last, level of milk production, use of towel, disinfecting milking material, drainage system, bedding material, injuries of udder and teat, teat blindness, tick infestation.

3.7. Data management and analysis

All data from laboratory tests and questionnaire surveys were entered into a Microsoft Excel spreadsheet and coded before performing statistical analysis. All the statistical analyses were performed using Stata software version 14.0. The dependent variable was the mastitis status of cows, and the potential risk factors considered as independent variables were age, breed,

parity, lactation stage, herd size, dry cow therapy, management system, washing udder before and after milking of an individual cow, floor type, previous mastitis history and milking mastitic cow last. An overall prevalence of mastitis was calculated by dividing the number of positive samples by the total number of animals sampled. The degree of association between the prevalence of mastitis and potential risk factors was evaluated by using odd ratio (OR) analysis. All variables with $p < 0.25$ in the initial univariable logistic regression analysis were checked for multicollinearity using Kruskal gamma statistics and those variables whose gamma value ranged between -0.6 and $+0.6$ were subjected to a multivariable logistic regression analysis. The final model was built in backward elimination of non-significant variables, and the model was evaluated for goodness-of-fit by using the Hosmer-Lemeshow method and for the predictive ability by using the receiver operating characteristic (ROC) curve (Dohoo *et al.*, 2009). In this analysis statistical significance was set at $p < 0.05$.

4. RESULTS

4.1. Prevalence of mastitis

An overall prevalence of mastitis at the cow level was 24.2% (92/380) with (95% CI: 20.1-28.8) from a total of 380 lactating cows examined for prevalence of mastitis. Of which 3.2% (12/380) was clinical mastitis and 21% (80/380) was subclinical mastitis (Table 3).

Table 3: Prevalence of mastitis at the cow and quarter-level

Observations	No. examined	Positive (n)		Prevalence (%)	95% CI
		Clinical (%)	Subclinical (%)		
Cow level	380	12 (3.2)	80 (21)	92 (24.2)	20.1-28.8
Quarter level	1,504	27 (1.8)	143 (9.5)	170 (11.3)	9.4-13.4

Out of 1,520 quarters examined for the existence of gross abnormalities, 16 (1.05%) quarters were blind. From the functional 1,504 quarters examined for mastitis, 170 (11.3%) with (95% CI: 9.4-13.4) were positive for mastitis (Table 4).

Table 4: Prevalence of mastitis at the quarter-level

Quarter	Quarters examined (n)	Functional quarters (n)	Blind quarter (n)	Positive (n)	Prevalence (%)
Right front	380	377	3	35	9.3
Right hind	380	374	6	51	13.6
Left front	380	378	2	37	9.8
Left hind	380	375	5	47	12.5
Total	1,520	1,504	16	170	11.3

Among selected kebeles from the study area for sample collection to estimate the prevalence of mastitis, the highest prevalence was recorded in Kebado town in both subclinical (35.1%) and clinical (5.4%) mastitis. However, there are no significant variations among the remaining kebeles (Table 5).

Table 5: Prevalence of mastitis by kebele

Kebele	Cows examined(n)	Subclinical		Clinical	
		Positive(n)	(%)	Positive(n)	(%)
Aleme-Korocho	62	11	16.1	2	3.2
Badessa	49	9	18.4	2	4.0
Gelowacho	48	9	18.8	1	2.1
Kebado	37	13	35.1	2	5.4
Korate	56	10	17.9	2	3.6
Machisho	53	10	18.9	2	3.8
Setamo	75	18	24.0	1	1.3
Total	380	80	21.0	12	3.2

4.2. Risk factors

Eleven possible risk factors were assessed for association between categories and the prevalence of mastitis using univariable logistic regression analysis (Table 6). The two variables, lactation stage, and floor type, were withdrawn from further analysis due to their statistically insignificant association ($p > 0.25$) with the presence of mastitis in the initial univariable logistic regression analysis. All variables with $p < 0.25$ in the initial univariable logistic regression analysis were checked for multicollinearity. The age of the cows was dropped from further analysis due to collinearity with parity ($\text{gamma} = -0.96$); parity was retained as a risk factor due to its important implications regarding mastitis and considered a more relevant variable than age based on biological plausibility. Thus, the variables subjected to multivariable logistic regression analysis were breed, parity, herd size, management system, dry cow therapy, washing udder, previous mastitis history, and milking mastitic cow last. Accordingly, the final logistic regression model revealed that breed, parity, management system, dry cow therapy, washing udder, and milking mastitic cow last remained significant ($p < 0.05$) predictors of mastitis in the cows. However, herd size and previous mastitis history had no significant association ($p > 0.05$) with the occurrence of mastitis in this study. The Hosmer-Lemeshow goodness-of-fit test revealed that the model fits the data ($\chi^2 = 5.02$; $p = 0.76$) (Table 7).

This study revealed that the breed of the cows significantly influence the prevalence of mastitis. A higher prevalence of mastitis (36.1%) was recorded in cross-breed cows compared to local breed cows (11.5%). The parity of the cows significantly influenced the occurrence of mastitis. The highest prevalence of mastitis (59.4%) was observed in cows that gave birth to 7

and above calves, followed by cows that gave birth to 4-6 calves (36.6%), and the lowest prevalence (14.4%) was recorded in cows that gave birth to 1-3 calves. There was also a significant difference in the prevalence of mastitis between different categories of management systems of cows. The prevalence of mastitis was found to be higher (47.1%) in cows that were kept under an intensive management system and lower prevalence (15.44%) in cows that were kept under an extensive management system. Statistical analysis also showed the existence of a significant difference between the presence of mastitis and dry cow therapy. The higher prevalence of mastitis (29.5%) was recorded in cows that were not treated during the dry period as compared to cows that were treated during the dry period (13.1%). In this study, washing udder revealed a statistically significant association with the prevalence of mastitis. The higher prevalence of mastitis (27.05%) was recorded in cows that were not washed udder and teat before milking compared to cows that were washed udder and teat before milking (18.94%). Milking mastitic cow last had a significant effect on the presence of mastitis. The prevalence of mastitis was found to be higher (30.24%) in cows from herds that did not practice milking mastitic cows last compared to cows from herds that did practice milking mastitic cows last (12.5%).

Table 6: Univariable logistic regression analysis for the association of different risk factors with the occurrence of mastitis

Risk factors	Cows examined (n)	Positive (n)	Prevalence (%)	OR	P value
Age					
Young (3-5 yrs)	79	28	35.4	Ref	
Adult (6-9 yrs)	182	41	22.5	0.53	0.031
Old (>9 yrs)	115	22	19.1	0.43	0.012
Breed					
Local	182	21	11.5	Ref	
Cross	194	70	36.1	4.33	0.000
Parity					
1-3 calves	243	35	14.4	Ref	
4-6 calve	101	37	36.6	3.44	0.000
>6 calve	32	19	59.4	8.69	0.000
Lactation stage					
Early	155	45	29	Ref	
Mid	129	33	25.6	0.79	0.377
Late	92	11	11.95	0.31	0.001
Herd size					
1-5	251	52	20.7	Ref	
>5	125	39	31.2	1.74	0.026
Management system					
Extensive	272	42	15.44	Ref	
Intensive	104	49	47.1	4.88	0.000
Dry cow therapy					
Yes	122	16	13.1	Ref	
No	254	75	29.5	2.78	0.001
Washing udder					
Yes	132	25	18.94	Ref	
No	244	66	27.05	1.59	0.081
Floor-type					
Concrete	80	20	25	Ref	
Wooden	282	41	22.5	0.87	0.663
Soil	114	30	26.3	1.07	0.837
Previous mastitis history					
Yes	231	45	19.5	Ref	
No	145	46	31.7	0.52	0.007
Milking mastitic cow last					
Yes	128	16	12.5	Ref	
No	248	75	30.24	3.03	0.000

Table 7: Multivariable logistic regression analysis for the association of different risk factors with the occurrence of mastitis

Variables	OR	95% CI	SE	Z	P value
Breed					
Local	Ref				
Cross	6.76	3.06-14.92	2.73	4.73	0.000
Parity					
1-3 calves	Ref				
4-6 calve	3.95	2.04-7.62	1.32	4.09	0.000
>6 calve	11.00	4.09-29.50	5.54	4.76	0.000
Herd size					
1-5	Ref				
>5	1.38	0.75-2.56	0.43	1.03	0.301
Management system					
Extensive	Ref				
Intensive	2.64	1.29-5.43	0.97	2.64	0.008
Dry cow therapy					
Yes	Ref				
No	2.65	1.32-5.34	0.95	2.74	0.006
Washing udder					
Yes	Ref				
No	2.86	1.44-5.68	1.00	3.01	0.003
Previous mastitis history					
Yes	Ref				
No	0.73	0.40-1.36	0.23	-0.98	0.329
Milking mastitic cow last					
Yes	Ref				
No	4.0	1.97- 8.08	1.44	3.85	0.000

4.3. Bacterial isolation

A total of 92 milk samples positive for mastitis at the cow level were cultured for bacteriological examination. Out of these, 13 (14.1%) milk samples did not show bacterial growth on culture media, whereas 79 (85.9%) samples showed growth with five groups of bacteria. Among these, 11(13.9%) were isolated from clinical mastitis and 68(86.1%) from subclinical mastitis. The predominant mastitis-causing bacterial pathogens isolated were *S. aureus* (32, 40.5%), followed by *Streptococcus* species (20, 25.3%), Coagulase-negative staphylococci (CNS) (13, 16.5%), *E. coli* (11, 13.9%) and *Klebsiella pneumoniae* (3, 3.8%) (Figure 3).

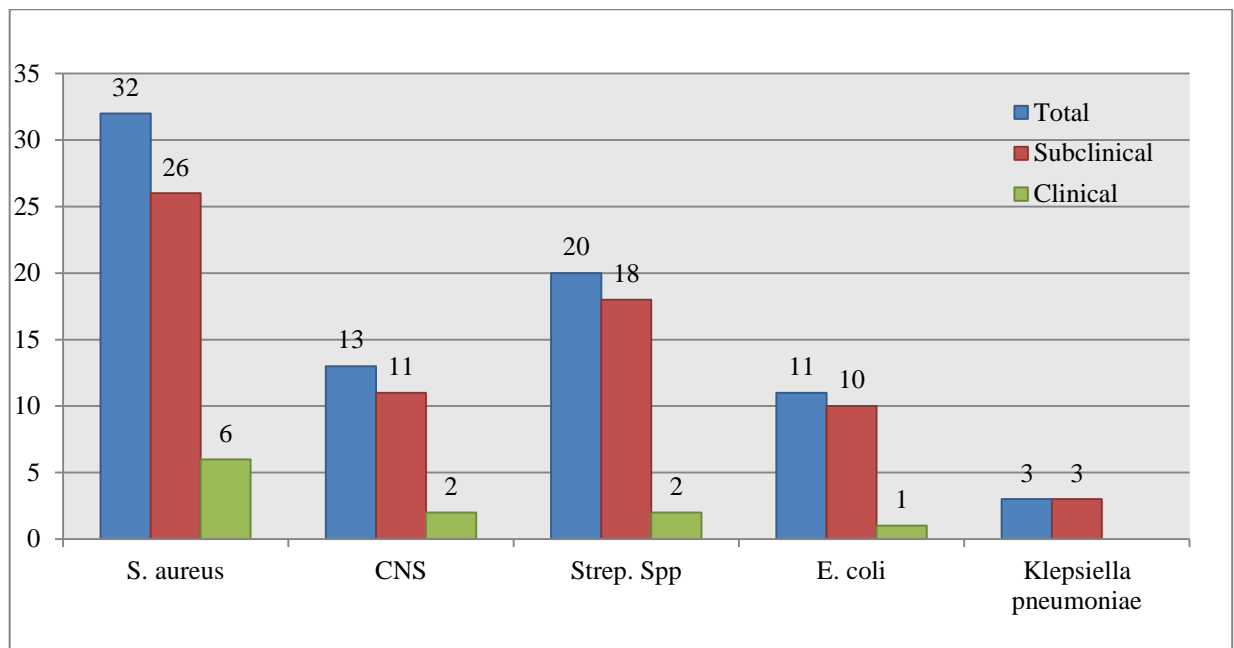


Figure 3: Bacterial isolates from milk samples

5. DISCUSSION

In the present study, the overall prevalence of mastitis in 380 lactating dairy cows in Dara district was 24.2%. This is in agreement with the prevalence reports of Yohannes and Alemu (2018) in Wolayta Sodo, Girma *et al.* (2012) in Doba district West-Hararghe, and Bitew *et al.* (2010) in Bahir Dar, who reported 24.5%, 23.18%, and 28.2%, respectively. However, it is higher than the other reports by Belay *et al.* (2022) in the Gamo zone and Anbu and Kumsa (2021) in and around Bedele town, who reported 17.1% and 18.1%, respectively. On the other hand, this finding is lower than higher prevalence reports of Fesseha *et al.* (2022) in Modjo town, Mekibib *et al.* (2010) in Holleta town, Tilahun and Aylate (2015) in Addis Abeba and Zenebe *et al.* (2014) in Adigrat, who reported 73.7%, 71%, 68%, and 64.3%, respectively. Variations in the prevalence of mastitis among different reports might be due to the complex nature of mastitis and its occurrence through the interactions of several factors, such as animal-level factors, environmental conditions, causative agents, and management and farming practices.

The prevalence of subclinical mastitis at 21% was higher than that of clinical mastitis at 3.2% in the present study. This is in agreement with several recent studies reported in different parts of Ethiopia by Bitew *et al.* (2010), Zenebe *et al.* (2014), and Tekle and Berihe (2016). The higher prevalence of subclinical might be attributed to little attention given to subclinical mastitis, because infected cows do not show visible signs of diseases and seem apparently healthy and continue to be a source of infection in the herd. In contrast, the efforts have been concentrated on the treatment of clinical cases (seid *et al.*, 2015).

An overall quarter-level prevalence of mastitis recorded in this study was 11.3%. This is in agreement with the finding of Girma *et al.* (2012) in Doba district of West-Harerghe who, reported 10.12%, but lower than the prevalence reports of Mekibib *et al.* (2010) in Holleta town and Fesseha *et al.* (2022) in Modjo town, who reported 44.9% and 36.9%, respectively. In the quarter-level prevalence of mastitis, the right hind quarter showed the relatively highest prevalence, 13.8%, followed by the left hind quarter, 12.4%. However, the front quarters showed a relatively lower prevalence, 9.9% in the left front quarter, and 9.1% in the right front quarter. This finding is in agreement with other similar reports by Abebe *et al.* (2016) in Hawassa and Zeryehun and Abera (2017) in the Eastern Harrarghe Zone. The higher prevalence of mastitis in hind quarters might be due to the hind quarters getting the first chance to contact the environment and feces, which exposes them to environmental contamination, hind quarters produce greater milk yield, and this tends to an increase the risk of mastitis with increase in milk production (Radostits *et al.*, 2007).

This study revealed that breed was significantly ($p < 0.05$) associated with the occurrence of bovine mastitis. The higher prevalence of mastitis observed in cross-breed cows were odds of 6.76 times more likely to have mastitis than in local breed cows. This is in line with other studies that reported similar findings Anbu and Kumsa (2021) in and around Bedele town, Abdeta and Gemechisa (2020) in Wolmara District, Oromia, Tsegaye *et al.* (2019) in Haramaya district, Kebebew and Jorga (2016) in Ambo town and Mitiku *et al.* (2017) in Sebeta, Central Ethiopia. This indicates that cross breeds are less resistant to the disease of mastitis compared to local (zebu) breeds. This might be because cross-breed cows have lower genetic potential for disease resistance and adaptability to the environment. Moreover, cross-

breed cows have giant udder and teat, which may give them more chance to contact a contaminated environment (Moges *et al.*, 2012).

This finding showed that parity was found to be strongly associated with the occurrence of bovine mastitis. The cows with many parity (>6 calves) were odds of 11 times more likely to have mastitis than cows with few parity (1-3 calves) or cows with several parity (4-6 calves) were odds of 3.95 times more likely to have mastitis than cows with few parity (1-3 calves).

This result is in agreement with the similar studies from various parts of the country reported by Bitew *et al.* (2010) in Bahir Dar, Tegegne *et al.* (2020) in central highlands of Ethiopia, and Abdeta and Gemechisa (2020) in Wolmara District. The higher prevalence of mastitis in cows with many parity (>6 calves) in this study is attributed to a decrease in defense mechanisms of cows through an increase in age. Repeated parturition also exposes cows to environmental and contagious mastitis-causing pathogens (Amin *et al.*, 2017).

The management system was significantly associated with the prevalence of mastitis in this finding. A higher prevalence of mastitis was observed in cows kept under intensive management systems were 2.64 times more likely to have mastitis than in cows kept under extensive management systems. This is in agreement with other reports of Ejeta *et al.* (2022) in and around Ambo town, and Tegegne *et al.* (2020) in the central highland of Ethiopia. This might be attributed to the difference in hygienic standards of dairy environment and milking practices. In most dairy farms, cows kept under an intensive system may confine in a dirty and wet area, which could favor the growth and transmission of mastitis-causing organisms (Tegegne *et al.*, 2020).

This study revealed that dry cow therapy was significantly associated with the prevalence of mastitis. A higher prevalence of mastitis was recorded in cows that did not receive treatment during the dry period and were 2.65 times more likely to have mastitis than cows that did receive treatment during the dry period. This could be due to dry cow therapy eliminating infectious agents present in the udder and preventing new infections during the dry period. This finding is in line with other studies reported by Zeryehun *et al.* (2013) in around Addis Ababa, Ethiopia.

The present study showed that washing the udder and teat before milking was significantly associated with the occurrence of bovine mastitis. Cows whose udder and teat did not wash before milking were 2.86 times more likely to have mastitis than cows whose udder and teat did wash before milking. Washing the udder and teat before milking is an essential activity for the reduction and prevention of the development of mastitis causing agents especially those that can be caused by environmental pathogens, and improves udder health. This finding is in agreement with the reports of Amin *et al.* (2017) in and around Haramaya town, and Lakew *et al.* (2009) in Asella.

Milking mastitic cow last was significantly associated with the occurrence of bovine mastitis. The prevalence of mastitis was odds of 4 times higher in cows from herds that did not milk mastitic cows last compared to those cows from herds that did milk mastitic cows last. The current result is in agreement with the findings of other report of Abebe *et al.* (2016) at the Hawassa milk shed, South Ethiopia. Lack of milk mastitic-cows last indicates that it could

facilitate the distribution of mastitis-causing pathogens within cows by the milker's hands and, consequently, the development of contagious mastitis (Belayneh *et al.*, 2013).

A total of 92 milk samples were cultured for bacteriological investigation in this study. Out of these, 79 (85.9%) samples have shown the growth of five groups of bacteria. Accordingly, the most prevalent bacteria isolates *S. aureus* 32 (40.5%), *Streptococcus* species 20 (25.3%), Coagulase negative *Staphylococcus* species 13 (16.5%), *E. coli* 11 (13.9%) and *Klebsiella pneumoniae* 3 (3.8%).

In this finding, *Staphylococcus* and *Streptococcus* were the two most dominant bacterial isolates with the proportion of *S. aureus* 32 (40.5%), *Streptococcus* species 20 (25.3%), and coagulase negative *Staphylococcus* species 13 (16.5%). This figure is in agreement with the studies of Tsegaye *et al.* (2019) in and around Haramaya district, eastern Ethiopia, Belayneh *et al.* (2013) in Adama-Ethiopia and Yohannis and Molla (2013) in and around Wolaita Sodo, Southern Ethiopia. The highest prevalence of *Staphylococcus* and *Streptococcus* might be due to lack of hand washing as well as udder and teat, absence of dry cow therapy, lack of milking mastitic cow last, absence of post milking teat dipping and disinfecting milking materials; as a result, pathogens can spread at the time of milking through milkers' hands and milking materials. In addition, the highest isolation of these bacteria might be due to their commensalism on the skin and mammary gland and localization on the injured skin of the teat and udder of cows (Abraham and Meaza, 2017).

In the present study, *E. coli* and *K. pneumoniae* were the coliform bacterial isolates with the prevalence of 13.9% and 3.8% respectively. These findings are in line with the reports of Belay *et al.* (2022) in the Gamo zone, Ethiopia, Fentahune *et al.* (2018) in and around Wukro of Tigray region, Ethiopia, and Mduhammad *et al.* (2013) in Pakistan. The high prevalence of these bacteria could be related to poor hygiene of environment and husbandry, poor sanitation of milking materials, absence of use of individual towels for each milking cow, and lack of dry cow therapy. Also, *E. coli* from fecal origin can contaminate the environment, and as a result, cause mastitis via bedding materials and milkers' hands (Heikkilä *et al.*, 2018).

6. CONCLUSION AND RECOMMENDATIONS

The present study indicated that mastitis is a major problem of dairy cows in the Dara district, which adversely affects the health and production of animals. Subclinical mastitis was a more prevalent type of mastitis compared to clinical mastitis. This indicates a lack of sufficient awareness of farmers about the impact of mastitis, especially subclinical type, lack of strategic control measures against the disease, and lack of maintenance of strict hygiene and sanitation of the animals and environment. Breed, parity, management system, dry cow therapy, washing udder and teat, and milking mastitic cows last were the major risk factors associated with the occurrence of mastitis. *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Streptococcus* species, *E coli*, and *K. pneumoniae* were the major bacterial isolates in the study area. Therefore, based on the study findings, the following recommendations are forwarded as a possible solution to prevent and control mastitis.

- ✚ Developing awareness of dairy farmers on the impact of mastitis, usage of dry cow therapy, and hygienic milking strategy.
- ✚ Improving the hygienic and sanitary status of the dairy cows, their environment, and materials.

Limitations of the study

Even though the study met its objectives, the limitations of this study were:

- ✓ Lack to conducting antimicrobial susceptibility tests on bacterial isolates to pay special attention to the challenges of drug resistance.
- ✓ Inability to identify streptococci and CNS at species level and failure to confirm using molecular test. Thus, these need to be studied for future investigation in this study area.

REFERENCES

- Abdali, F., Hosseinzadeh, S., Berizi, E. and Pourmontaseri, M. (2020). Prevalence of *Brucella* species in unpasteurized dairy Products consumed in Shiraz province using PCR assay. *Molecular Biology Research Communications*; **9** (3): 117-121.
- Abdeta, D and Gemechisa, B. (2020). A Study on the Prevalence of Subclinical Mastitis in Lactating Cows and Associated Risk Factors in Wolmara District, Oromia Regional State, Ethiopia. *Biomedical Journal of Scientific and Technical Research*; **28** (2): 21421-21426.
- Abebe, R., Hatiya, H., Abera, M., Megersa, B. and Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*; **12** (270): 1-11.
- Abera, B., Lemma, D. and Iticha, I. (2013). Study of bovine mastitis in Asella government dairy farm of Oromia Regional state, South Eastern Ethiopia. *International Journal of Current Research and Academic Review*; **1** (2): 134-145.
- Abera, M., Elias, B., Aragaw, K., Denberga, Y., Amenu, K. and Desie, S. (2012). Major causes of mastitis and associated risk factors in smallholder dairy cows in Shashemene, southern Ethiopia. *African Journal of Agricultural Research*; **7** (24): 3513-3518.
- Abraham, F. and Meaza, M. Z. (2017). Prevalence of Bovine Clinical Mastitis and Farmer's Awareness in and Around Wolaita Sodo, Southern Ethiopia. *Advances in Dairy Research*; **5** (3): 1-6.

- Abunna, F., Fufa, G., Megersa, B. and Regassa, A. (2013). Bovine Mastitis: Prevalence, Risk Factors and Bacterial Isolation in Small-Holder Dairy Farms in Addis Ababa City, Ethiopia. *Global Veterinary*; **10** (6): 647-652.
- Adane, B., Guyo, K., Tekle, Y., Taddele, H., Bogale, A. and Biffa, D. (2012). Study on Prevalence and Risk Factors of Bovine Mastitis in Borana Pastoral and Agro-Pastoral Settings of Yabello District, Borana Zone, Southern Ethiopia. *American-Eurasian Journal of Agriculture and Environment Science*; **12** (10): 1274-1281.
- Adkins, P. R. and Middleton, J. R. (2018). Methods for diagnosing mastitis. *Veterinary Clinic North America Food Animal Practice*; **34**: 479-91.
- Alemu, S., Tamiru, F., Almaw, G. and Tsega, A. (2013). Study on bovine mastitis and its effect on chemical composition of milk in and around Gondar Town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*; **5** (8): 215-221.
- Amin, B., Deneke, Y. and Abdela, N. (2017). Bovine Mastitis: Prevalence, Risk Factors and Isolation of *Streptococcus* Species from Small Holders Dairy Farms in and Around Haramaya Town, Eastern Ethiopia. *Global Journal of Medical Research: Microbiology and Pathology*; **17** (1): 27-38
- Anbu, E. G. and Kumsa, K.M. (2021). Prevalence of bovine mastitis and its associated risk factors in and Around Bedele Town. *Epidemiology International Journal*; **5** (3): 194.
- Ararsa, D, Tadele, T. and Aster, Y. (2014). Prevalence of clinical and subclinical mastitis on cross-breed dairy cows at Holleta Agricultural Research Center, Central Ethiopia. *Journal of Veterinary Medicine and Animal Health*; **6**: 13-17.

- Atasever, S. and Erdem, H. (2009). Association between subclinical mastitis markers and body condition scores of Holstein cows in the Black Sea region, Turkey. *Journal of Animal and Veterinary Advances*; **8**: 476- 480.
- Bachaya, H. A., Raza, M. A., Murtaza, S. and Akbar, I. U. (2011). Subclinical bovine mastitis in Muzaffar Garh district of Punjab, Pakistan. *Journal of Animal and Plant Sciences*; **21** (1): 16-19.
- Belay, N., Mohammed, N. and Seyoum, W. (2022). Bovine Mastitis: Prevalence, Risk Factors, and Bacterial Pathogens Isolated in Lactating Cows in the Gamo Zone, Southern Ethiopia. *Journal of Veterinary Medicine: Research and Reports*; **13**: 9-19.
- Belayneh, R., Belihu, K. and Wubete, A. (2013). Dairy cows mastitis survey in Adama Town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*; **5** (10): 281-287.
- Beyene, B. and Tolosa, T. (2017). Epidemiology and financial impact of bovine mastitis in an animal production and research center and smallholder dairy farms in Horoguduru Wollega zone, western Ethiopia. *Journal of Dairy, Veterinary and Animal Research*; **5** (4): 144-151.
- Birhanu, M., Leta, S., Mamo, G. and Tesfaye, S. (2017). Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. *BMC Research Notes*; **10** (1): 1-6.
- Bitew, M., Tolosa, T. and Tefera, A. (2010). Study on Bovine Mastitis in Dairy Farms of Bahir Dar and its Environments. *Journal of Animal and Veterinary Advances*; **9** (23): 2912-2917.

- Bytyqi, H., Zaugg, S., Hamidi, G. and Muji. (2010). Influence of management and physiological factors on somatic cell count in raw milk in Kosova. *Veterinary Archive*; **80** (2):173-183.
- Cervinkova, D., Vlkova, H., Borodacova, I., Makovcova, J., Babak ,V., Lorencova, A., Vrtkova, I., Marosevic, D. and Jaglic, Z. (2013). Prevalence of mastitis pathogens in milk from clinically healthy cows. *Journal of Veterinary Medicine*; **58** (11): 567-575.
- Cheng, D., Yuan, S. Z., Hua, Z. Y., Wei, W. D., Xia, Z. M., Rui, Z. S. and Chang, H. S. (2010). Prevalence of bacterial infection responsible for bovine mastitis. *African Journal of Microbiology Research*; **4** (11):1110-1116.
- Cheng, W. N. and Han, S. G. (2020). Bovine mastitis: Risk factors, therapeutic strategies, and alternative treatment. *Asian-Australian Journal of Animal Science*; **33** (11): 1699-1713.
- CSA. (2020). Agricultural sample survey. Report on livestock and livestock characteristics (private peasant holdings). Federal Democratic Republic of Ethiopia CSA, Addis Ababa. *Statistical Bulletin*; 587 (2).
- Dara District Finance and Economy Development Office. (2021). Socio-economic and geo-referenced data analysis and distribution department. Kebado. Unpublished
- Dara District Livestock Resource Development Office. (2022). Livestock extension communication department. Kebado. Unpublished
- Deng, Y., Tamir, B. and Asebe, G. (2015). Assessment of hygienic milk production and prevalence of mastitis in dairy cows in Jikawo Woreda of Nuer Zone, Gambella-Region, Ethiopia. *Journal of Agriculture and Natural Resources Sciences*; **2**: 480-486.

- DeVliegher, S., Fox, L. K., Piepers, S., McDougall, S. and Barkema, H. W. (2012). Mastitis in dairy heifers: Nature of the disease, potential impact, prevention and control. *Journal of Dairy Science*; **95** (3): 1025-1040.
- Dohoo, I., Martin, W. and Stryhn, H. (2009). *Veterinary Epidemiologic Research*. 2nd edition. Charlottetown: AVC Inc; pp. 27-407.
- Durr, J., Cue, R., Monardes, H., MoroMéndez, J. and Wade, K. (2008). Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. *Livestock Science*; **117**: 225- 232.
- Ejeta, I., Serda, B. and Pal, M. (2022). Studies on the prevalence of bovine mastitis and its associated risk factors in and around Ambo Town Oromia Region, Central Ethiopia. *International Journal of Livestock Research*; **12** (3): 15-24.
- Elango, A., Doraisamy, K.A., Rajarajan, G. and Kumaresan. G. (2010). Bacteriology of subclinical mastitis and antibiogram of isolates recovered from cross-breed cows. *Indian Journal of Animal Research*; **44** (4): 280-284.
- Etifu, M. and Tilahun, M. (2019). Prevalence of bovine mastitis, risk factors, isolation and anti-biogram of major pathogens in Mid Rift Valley, Ethiopia. *International Journal of Livestock Production*; **10** (1): 14-23.
- Fadlelmula, A., Dughaym, A. M., Mohamed, G. E., Deib, M. K. and Zubaidy, A. J. (2009). Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. *Bulgarian Journal of Veterinary Medicine*; **12**: 199-206.
- FAO. (2014). Impact of mastitis in small-scale dairy production systems. Animal Production and Health Working Paper. Rome; No. 13.

- Fentahun, T. D., Taddele, H. M. and Adugna, M. M. (2018). Prevalence of mastitis and identification of its bacterial causative agents in small holder dairy farms in and around Wukro of Tigray region, Ethiopia. *International Journal of Advanced Research in Biological sciences*; **5** (11): 10-22.
- Fesseha, H., Mathewos ,M., Aliye, S. and Wolde, A. (2022). Study on Prevalence of Bovine Mastitis and Associated Risk Factors in Dairy Farms of Modjo Town and Suburbs, Central Oromia, Ethiopia. *Veterinary Medicine: Research and Reports*; **12**: 271-283.
- G/Michael, L., Deressa, B., Begna, F. and Mekuria, A. (2013). Study on prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka Town, Southern Ethiopia. *African Journal of Microbiology Research*; **7** (43): 5051-5056.
- Galal, P., Bassili, A., Grant, A. D., El-Mohgazy, E., Glaziou, A., Seita, I., Bierrenbach, J. P., Crofts, N. A. and Van, H. (2007). Estimating tuberculosis case detection rate in resource-limited countries: A capture-recapture study in Egypt. *International Journal of Tuberculosis and Lung Disease*; **14** (6): 727-732.
- Getahun, K., Kelay, B., Bekana, M. and Lobago, F. (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Tropical Animal Health Production*; **40**: 261-268.
- Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. and Jibat, T. (2012). Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *Journal of Veterinary Medicine and Animal Health*; **4** (8): 116-123.
- Giuliano, C., Patel, C. and Kale-Pradhan, P. (2019). A guide to bacterial culture identification and results interpretation. *Pharmacy and Therapeutics*; **44** (4): 192.

- Guadu, T. and Abebaw, M. (2016). Challenges, Opportunities, and Prospects of Dairy Farming in Ethiopia. *World Journal of Dairy and Food Sciences*; **11** (1): 01-09.
- Haftu, R., Taddele, H., Gugsu, G. and Kalayou, S. (2012). Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. *Tropical Animal Health and Production*; **44**: 1765-1771.
- Halasa, T., Nielen, M., De Roos, A. P., Van Hoorne, R., de Jong, G., Lam, T. J., van Werven, T. and Hogeveen, H. (2009). Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *Journal of Dairy Science*; **92** (2): 599-606.
- Hassanain, A., Soliman, A., Ghazy, A. and Ghazyi, A. (2009). Bovine tuberculosis in a dairy cattle farm as a threat to public health. *African Journal of Microbiology Research*; **3** (8): 446-450.
- Heikkilä, A. M., Liski, E., Pyörälä, S. and Taponen, S. (2018). Pathogen-specific production losses in bovine mastitis. *Journal of Dairy Science*; **101** (10): 9493-9504.
- Hossain, M., Paul, S., Islam, M. and Alam, M. (2017). Bovine mastitis and its therapeutic strategy during an antibiotic sensitivity test. *Austin Journal of Veterinary Science and Animal Husbandry*; **4** (1): 1030.
- Huijps, K., Lam, T. J. and Hogeveen, H. (2008). Costs of mastitis: facts and perception. *Journal of Dairy Research*; **75**: 113-120.
- Islam, M. A., Rahman, A.K., Rony, S. A. and Islam, M. S. (2010). Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. *Bangladesh Journal of Veterinary Medicine*; **8** (2): 157-162.
- Ismael, A. (2018). Epidemiology of Bovine Mastitis in Ethiopia. A review article. *Journal of Veterinary Medicine and Health*; **2** (1): 1-7.

- Jones, G. M. (2009). Proper Dry Cow Management is Critical for Mastitis Control. Virginia Polytechnic Institute and State University, College of Agriculture and Life Sciences. pp 404-412.
- Juliana, L., Maria, A., Carla, C., Marcio, R., Jo'ao, B., Let'icia, C., Juliana, F. and Guilherme, N. (2017). Estimate of the economic impact of mastitis: a case study in a Holstein dairy herd under tropical conditions. *Journal of Preventive Medicine*; 4231.
- Katsande, S., Matope, G., Ndengu, M. and Pfukenyi, D. M. (2013). Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort Journal of Veterinary Research*; **80**: 523.
- Kebebew, G. and Jorga, E. 2016. Prevalence and risk factors of bovine mastitis in Ambo Town of West Shewa Zone, Oromia, Ethiopia. *Ethiopian Veterinary Journal*; **20** (1):123-134.
- Kemp, M. H., Nolan, A. M., Cripps, P. J. and Fitzpatrick, J. L. (2008). Animal-based measurements of the severity of mastitis in dairy cows. *Veterinary Records*; **163**: 175-179.
- Król, J., Brodziak, A., Litwińczuk, Z. and Litwińczuk, A. (2013). Effect of age and stage of lactation on whey protein content in milk of cows of different breeds. *Pol. J. Vet. Sci.*; **16** (2): 395-397.
- Kumar, M., Goel, P., Sharma, A. and Kumar, A. (2009). Prevalence of subclinical mastitis in cows at a Goshala. Proceedings of Compendium of 27th edition ISVM. International Summit and Convention at Chennai, Tamilnadu, India. pp 4-7.

- Lakew, M., Tolosa, T. and Tigre, W. (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South-Eastern, Ethiopia. *Tropical Animal Health and Production*; **41**: 1525-1530.
- Lam, T., Olde, Riekerink, R., Sampimon, O., and Smith, H. (2009). Mastitis diagnostics and performance monitoring: A practical approach. *Irish Veterinary Journal*; **62** (4): 120-128.
- Lijalem, T., Asefa, A. and Sharo, A. (2015). Challenges and Opportunities of Dairy Cattle Production in Selected Districts of Sidama Zone, Southern Ethiopia. *Food Science and Quality Management*; **44**: 1-12.
- Mahantesh. M. Kurjogi and Basappa. B. Kaliwal. (2011). Prevalence and Antimicrobial Susceptibility of Bacteria Isolated From Bovine Mastitis. Pelagia Research Library. *Advances in Applied Science Research*; **2** (6): 229-235.
- Mahmmod, Y. (2013). The Future of PCR Technologies in Diagnosis of Bovine Mastitis Pathogens. *Advances in Dairy Research*; **2** (1): 1-6.
- Manning, S. D., Springman, A. C., Million, A. D., Milton, N. R., MacNamara, S. E., Somsel, P. A., Bartlett, P. and Davies, H. D. (2010). Association of group B *Streptococcus* colonization and bovine exposure. PLoS ONE; **5** (1): 1-6.
- Matios, L., Tadele, T. and Worku, T. (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical Animal Health and Production*; **41**: 1525-1530.
- Mekibib, B., Furgasa, M., Abunna, F., Megersa, B. and Regassa, A. (2010). Bovine Mastitis: Prevalence, Risk Factors and Major Pathogens in Dairy Farms of Holeta Town, Central Ethiopia. *Veterinary World*; **3** (9): 397-403.

- Mekonnen, E., Eshetu, E., Awekew, A. and Thomas, N. (2016). A Study on the Prevalence of Bovine Mastitis and Associated Risk Factors in and the Surrounding Areas of Sodo Town, Wolaita Zone, Ethiopia. *Global Journal of Science Frontier Research: Agriculture and Veterinary*; **16** (2): 13-20.
- Abebe, B. and Bakala, S. (2022). Prevalence: Bovine mastitis and its predisposing factors in and around Holeta Town, Oromia, Ethiopia. *International Journal of Veterinary Science and Research* **8** (4): 151-159.
- Mitiku, Y., Gizaw, Y. and Kassa, T. (2017). The Prevalence of Bovine Mastitis and Associated Risk Factors in Cross Breed Lactating Dairy Cows in Sebeta, Central-Ethiopia. *European Journal of Biological Sciences*; **9** (3): 106-112.
- Moges, N., Hailemariam, T., Fentahun, T., Chanie, M. and Melaku, A. (2012). Bovine mastitis and associated risk factors in small-holder lactating dairy farms in Hawassa, Southern Ethiopia. *Global Veterinary*; **9** (4): 441-446.
- Muhammad, N., Baharullah, K., Abdul, S., Taiseer, I., Qaiser, J. and Shahzad, M. (2013). Biochemical and Bacteriological Analysis of Cows' Milk Samples Collected from District Peshawar. *International Journal of Pharmaceutical Sciences Review and Research*; **21** (2): 221-226.
- NAAS. (2013). Mastitis Management in Dairy Animals. Policy Paper: *National Academy of Agricultural Sciences, New Delhi*; **61**:12-36.
- National Mastitis Council. (2004). Microbiological procedures for the diagnosis of bovine udder infection, 3rd edition. Madison, Wisconsin; pp 7-8.
- Olde, R. R., Barkema, H. W., Kelton, D. F. and Scholl, D. T. (2008). Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science*; **91** (4): 1366-1377.

- Olivares-Pérez, J., Kholif, A. and Rojas, H. (2015). Prevalence of bovine subclinical mastitis, its etiology, and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico. *Tropical Animal Health and Production*; **47**: 1497-1504.
- Ondiek, O., Ogore, P., Shakala, E., and Kaburu, G. (2013). Prevalence of bovine mastitis, its therapeutics, and control in Tatton Agriculture Park, Egerton University, Njoro District of Kenya. *Basic Research Journal of Agricultural Science and Review*; **2** (1):15-20.
- Pal, M. (2018). Mastitis: A major production disease of dairy animals. *Agriculture World*; **4**: 46-51.
- Pandey, V., Aditi, P., Gupta, S., Neelesh, S. and Deepak S. (2012). Impact of subclinical mastitis on blood biochemistry of dairy cows. *The Indian Journal of Animal Sciences*; **82** (5): 477-478.
- Persson, W., Bengtsson, K., Lindberg, B., Nyman, A. and Ericsson, U. (2009). Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows- influence of breed and stage of lactation. *Veterinary Microbiology*; **134**: 89-94.
- Petzer, I. M., Karzis, J., Donkin, E. F., Webb, E. C. and Etter, E. M. (2016). Pathogen-specific approach towards udder health management in dairy herds using micro-cytology from routine herd investigations. *Onderstepoort Journal of Veterinary Research*; **83** (1): 1146.
- Qolbaini, N., Artika, M. and Safari, D. (2014). Detection of Subclinical Mastitis in Dairy Cows using California Mastitis Test and Udder Pathogen. *Current Biochemistry*; **1** (2): 66 - 70.

- Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (2004). *Clinical Veterinary Microbiology*. Mosby International Limited, London. pp 327-344.
- Radostits, O. M, Gay, C. C., Hinchcliff, K. W. and Constable, P. D. (2007). *Veterinary Medicine: A Text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. 10th edition. London: Elsevier. pp 674-762.
- Ravea, A. F., Kussb, A. V., Peila, G. H., Ladeirac, S. R., Villarreal, J. P. and Nascentee, P. S. (2019). Biochemical identification techniques and antibiotic susceptibility profile of lipolytic ambient bacteria from effluents. *Brazilian Journal of Biology*; **79** (4); 555-565.
- Roberson, J. R. (2012). Treatment of Clinical Mastitis. *Veterinary Clinic North America: Food Animal Practice*; **28**: 271-288.
- Sadek, K., Saleh, E. and Ayoub, M. (2017). Selective, reliable blood and milk bio-markers for diagnosing clinical and subclinical bovine mastitis. *Tropical Animal Health and Production*; **49**: 431-437.
- Saidi, R., Khelef, D., and Kaidi, R. (2013). Bovine mastitis: Prevalence of bacterial pathogens and evaluation of early screening test. *African Journal of Microbiology Research*; **7** (9): 777-782.
- Sayeed, A., Rahman, A., Bari, S., Islam, A., Rahman, M. and Hoque, A. (2020). Prevalence of Subclinical Mastitis and Associated Risk Factors at Cow Level in Dairy Farms in Jhenaidah, Bangladesh. *Advances in Animal and Veterinary Sciences*; **8** (2): 112-121.
- Seid U., Zenebe, T., Almaw, G., Edao, A., Disassa, H., Kabeta, T., Gerbi, F. and Kebede G. (2015). Prevalence, risk factors and major bacterial causes of bovine mastitis in West Arsi Zone of Oromia Region, Southern Ethiopia. *Natural Science*; **13**: 19-27.

- Sharif, A. and Muhammed, G. (2009). Mastitis control in dairy animals. *Pakistan Veterinary Journal*; **29** (3): 145-148.
- Sharma, N., Singh, K. and Bhadwal, S. (2011). Relationship of Somatic Cell Count and Mastitis: An Overview. *Asian-Australians Journal of Animal Science*; **24** (3): 429-438.
- Shiferaw, E. (2009). Bovine mastitis in the dairy farms of the eastern part of Amhara region, Ethiopia: Industrious problem. *Ethiopian Veterinary Journal*; **13**: 1-8.
- Shiferaw, J. and Telila, I. (2016). Prevalence of Bovine Mastitis and Assessment of Risk Factors in and Around Wolayta Sodo, Ethiopia. *International Journal of Homeopathy & Natural Medicines*; **2** (1): 1-7.
- Singh, D., Kumar, S., Singh, B. and Bardhan, D. (2014). Economic losses due to important diseases of bovines in central India. *Veterinary World*; **7** (8): 579-585.
- Sumathi, B.R., Veeregowda, B.M. and Amitha, R. G. (2008). Prevalence and antibiogram profile of bacterial Isolates from clinical bovine mastitis. *Veterinary World*; **1** (8): 237-238
- Taponen, S., Salmikivi, L., Simonjoki, H., Koskinen, M. T. and Pyörälä, S. (2009). Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. *Journal of Dairy Science*; **92**: 2610-2617.
- Tegegne, D.T., Yalew, S.T., Emeru, B.A., Messele, Y.E., Werid, G.M., Bora, S.K. and Babura M.D. (2020). Study of Prevalence, Associated Risk Factors and Causative Bacteria of Bovine Mastitis in Ethiopia. *International Journal of Veterinary Science and Technology*; **4** (1): 001-006.

- Tekle, Y. and Berihe, T. (2016). Bovine mastitis: prevalence, risk factors and major pathogens in the Sidama Zone SNNPRS, Ethiopia. *European Journal of Biology and Medical Science Research*; **4**: 27-43.
- Tesfaye, B. (2016). Bovine mastitis: prevalence, isolation of bacterial species involved and its Antimicrobial susceptibility test around Debrezeit, Ethiopia. *Journal of Veterinary Science Technology*; **7**: 396.
- Tezera, M. and Aman, E. (2021). Prevalence and associated risk factors of bovine mastitis in dairy cows in and around Assosa Town, Benishangul-Gumuz Regional State, Western Ethiopia. *Veterinary Medicine and Science*; **7** (4): 1280-1286.
- Thrusfield, M., Christley, R., Brown, H., Peter, J., French, N., Howe, K., Kelly, L., O'Connor, A., Sarge, J. and Wood, H. (2018). *Veterinary Epidemiology*, 4th Edition. Royal School of Veterinary Studies University of Edinburgh: Wiley Blackwell; 276.
- Tilahun, A. and Aylate, A. (2015). Prevalence of Bovine Mastitis in Lactating Cows and its Public Health Implications in Selected Commercial Dairy Farms of Addis Ababa. *Global Journal of Medical Research: Veterinary Science and Veterinary Medicine*; **15** (2): 17-24.
- Tolosa, T., Verbeke, J., Piepers, S., Supré, K. and De Vlieghe, S. (2013). Risk factors associated with subclinical mastitis as detected by California Mastitis Test in smallholder dairy farms in Jimma, Ethiopia, using multilevel modeling. *Preventive Veterinary Medicine*; **112**: 68-75.
- Tsegaye, B.L., Fayera, T. and Muktar, Y.A. (2019). Risk factors for bovine mastitis with the isolation and identification of *Streptococcus agalactiae* from farms in and around

- Haramaya district, eastern Ethiopia. *Tropical Animal Health and Production*; **51**:1507-1513.
- Verma, H., Rawat, S., Sharma, N., Jaiswal, V. and Singh R. (2018). Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. *Journal of Entomology and Zoology Studies*; **6** (1): 706-709.
- Yohannes, K. and Alemu, B. (2018). Prevalence of Bovine Mastitis in lactating Cows and Associated risk factors in and around Wolayta Soddo, Southern Ethiopia. *International Journal of Advanced Research in Biological Sciences*; **5** (12): 60-69.
- Yohannis, M. and Molla, W. (2013). Prevalence, risk factors and major bacterial causes of bovine mastitis in and around Wolaita Sodo, Southern Ethiopia. *African Journal of Microbiology Research*; **7** (48): 5400-5405.
- Yusuf, M. and Husen, M. (2023). Prevalence and Incidence of Bovine Mastitis in Dairy Farm of Haramaya University, Eastern Ethiopia. *Journal of Veterinary Science and Animal Husbandry*; **10** (3): 1120.
- Zadoks, R. N., Middleton, J. R., McDougall, S., Katholm, J. and Schukken, Y. H. (2011). Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *J. Mammary Gland Biol. Neoplasia*; **16**: 357-372.
- Zenebe, N., Habtamu, T. and Endale, B. (2014). Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *African Journal of Microbiology Research*; **8** (4): 327-331.

ANNEXES

Annex I: Questionnaire survey format

Code _____

Date _____

A. Owner Identification

Owner's name _____ Sex: Male _____ Female _____

District _____ Kebele _____ Village _____

Educational background of the farm owner/attendant:

- | | |
|----------------------------|---------------------------------|
| a) Illiterate | b) Primary School (1-8) |
| c) Secondary school (9-12) | d) College /University graduate |

B. Animal data

1. Dairy herd size: _____

Number of lactating cows _____ Number of dry cows _____ No of heifers _____

Number of bulls _____ Number of calves _____

- | | | | |
|---------------------------------|-----------------|--------------|--------------|
| 2. Breed of lactating cows: | a) Local (zebu) | b) Cross | |
| 3. Lactation stage in months | a) Early (1-4) | b) Mid (5-8) | c) Late (>8) |
| 4. Parity of the lactating cows | a) 1-2 calves | b) 3-6calves | c) >6calves |
| 5. Age in years | a) 2-4 | b) 5-7 | c) ≥ 8 |

6. Udder and teat hygiene

- | | | |
|---|--------|-------|
| • Do you wash the udder before milking? | a) Yes | b) No |
| • Do you dry after washing? | a) Yes | b) No |
| • Do you use the same cloth for all cows? | a) Yes | b) No |
| • Do you practice milking mastitis cows last? | a) Yes | b) No |

- Do you dipping teats before milking? a) Yes b) No
- Do you dipping teats after milking? a) Yes b) No)
- 7. Do you practice dry cow therapy a) Yes b) No
- 8. Tick infestation: a) Absent b) Present
- 9. Teat lesion: a) present b) absent

10. Housing System

- House type: a) Fenced stable b) House barn
- Slop: a) sloppy b) leveled
- Roof: a) metal sheet b) grass
- Floor type: a) concrete b) stone c) soil
- Wall: a) concrete b) mud c) others (specify) _____
- Manure removal: a) daily b) weekly c) monthly d) other (specify) _____

11. Management system a) Intensive b) Extensive

12. Grazing system a) Communal b) Private c) Both

Do you move your herds to other places for grazing seasonally? a) Yes b) No

If yes,

When (season)? _____

Where? _____

How long did you keep them there?

13. Culling chronically infected cows a) Yes b) No

C. Knowledge of mastitis

1. Have you ever hear about mastitis? a) Yes b) No
2. From who did you heard?
a) Parents b) Friend(s) c) Health workers d) Media
3. Can poor hygienic management result in mastitis problems in this area?
a) Yes b) No I don't know
4. What are the common clinical signs seen?

5. Can mastitis reduce a milk yield? a) Yes b) No
If yes, how much amount in liters/day? _____
6. Is mastitis treatable? a) Yes b) No c) I don't know
7. Types of treatment sought for animals
a) traditional/herbal medicines b) chemical drugs
8. Is a mastitis disease is curable? a) Yes b) No c) I don't know
9. Have you encountered mastitis on your farm a) Yes b) No
10. Which breed is more susceptibility? a) Local b) Cross
11. At what stage of lactation do you observe?
a) At early-stage b) At mid-stage c) At late-stage
12. At what parity level become more susceptible a) 1-2 b) 3-6 c) >6
13. Effect of mastitis on milk production and productivity
a) Decreases milk quality b) Decreases yield c) Both

Annex III: Figures for CMT test and laboratory examination of milk sample

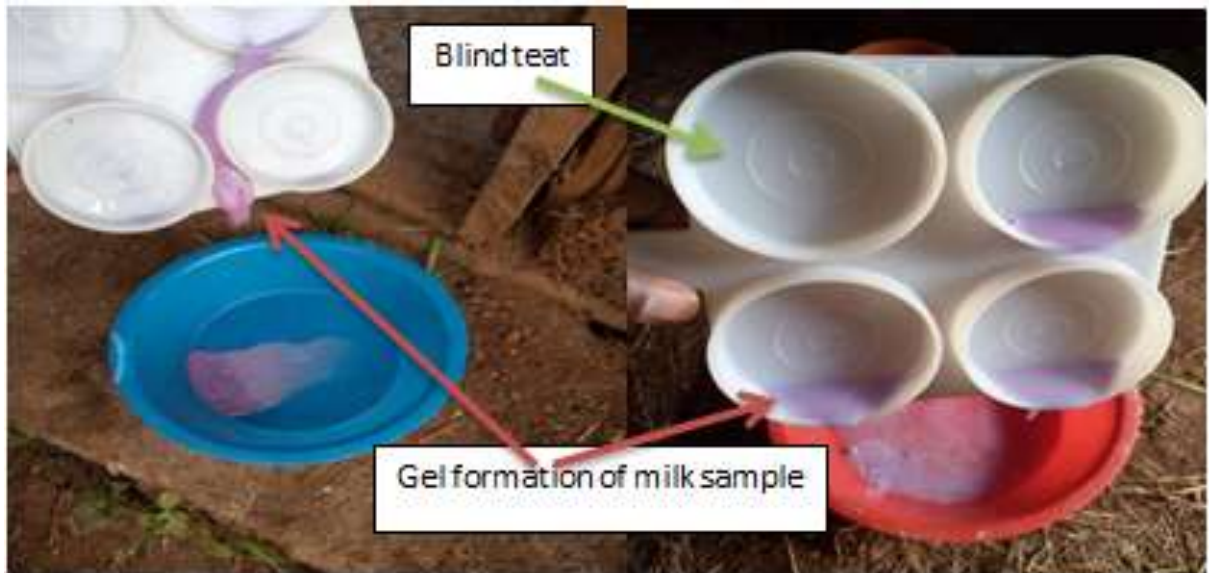


Figure 1: CMT test result

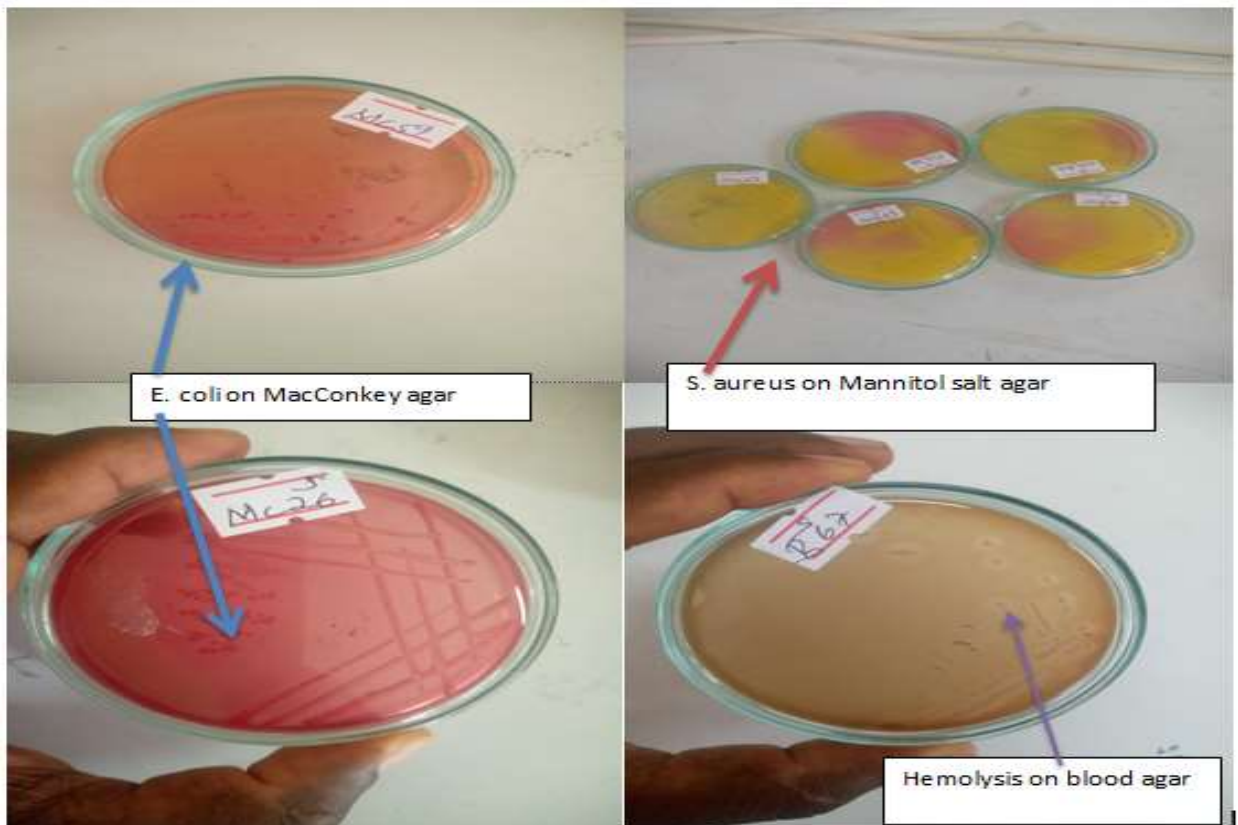


Figure 2: Colony morphology of bacteria on different culture media

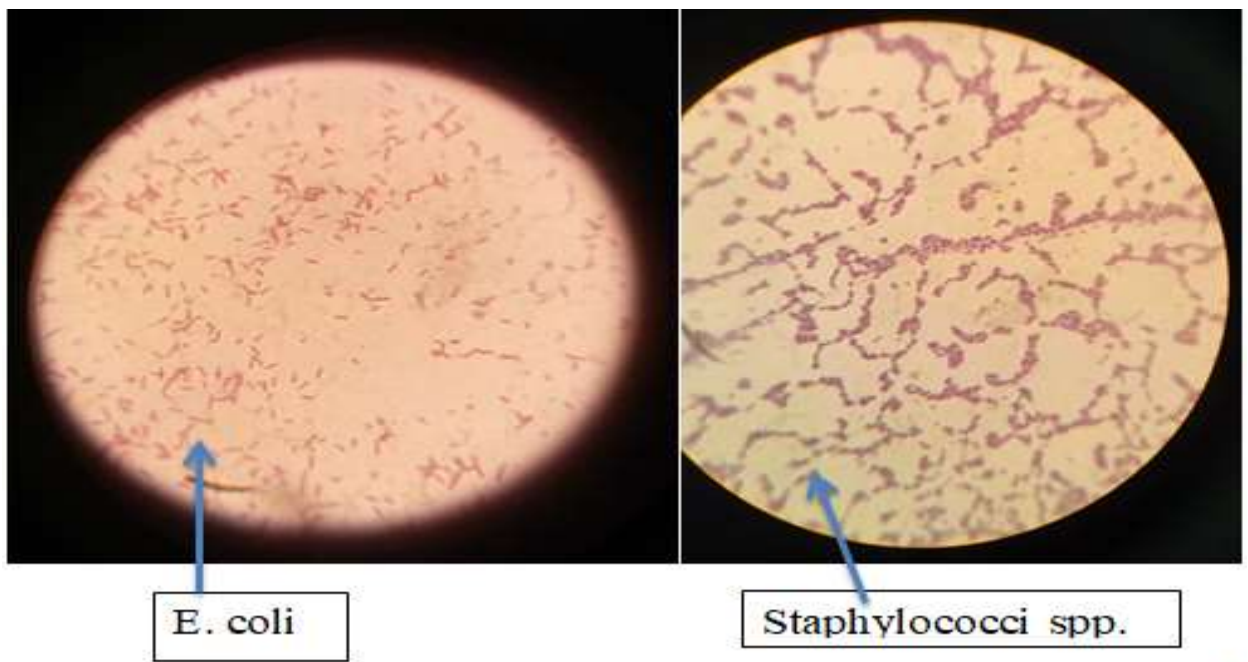


Figure 3: Gram staining results

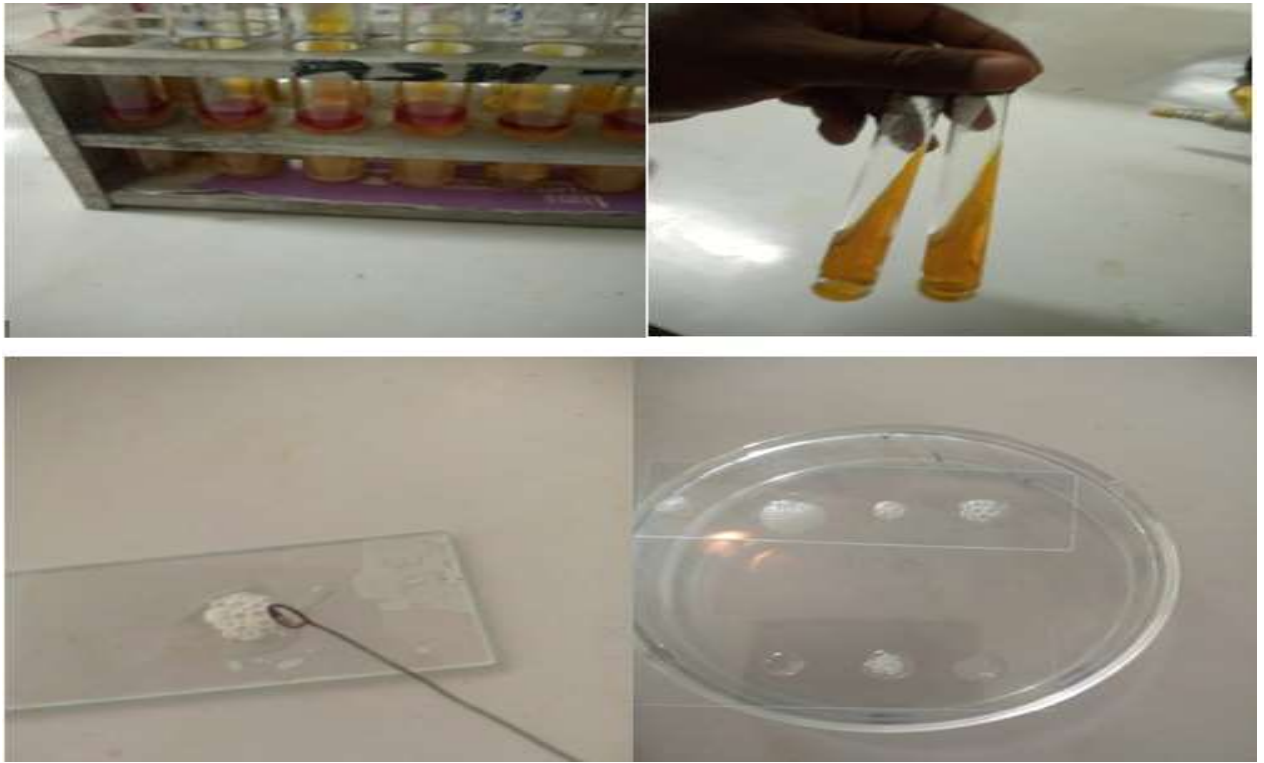


Figure 4: Biochemical tests results