

**ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**STUDY ON PREVALENCE OF MASTITIS AND ASSOCIATED RISK FACTORS
WITH ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF MAJOR
PATHOGENS IN ALAGE STATE DAIRY FARM, ETHIOPIA**

BY

MELESSE ETIFU MERANGA

JUNE, 2012

DEBRE ZEIT ETHIOPIA

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LIST OF ABBREVIATIONS

A.A.T.V.E.T.C.	Alage Agriculture Technical Vocational Education Training College
AM	Anti Meridian
CM	Clinical Mastitis
CMT	California Mastitis Test
IDF	International Dairy Federation
IMI	Intra Mammary Infection
OIE	Office International des Epizooties
PM	Post Meridian
SCC	Somatic Cell Count
SCM	Subclinical Mastitis

ABSTRACT

Cross-sectional study design was implemented in Alage dairy farm from September 2011 to April 2012 to determine the overall prevalence of mastitis, isolation of mastitis pathogens with their anti-biogram susceptibility patterns and risk factors to mastitis were also investigated. 444 quarter of 111 exotic dairy cows were examined. Overall prevalence of mastitis at cow and quarter level was found to be 73% and 37% respectively. Among 444 quarters examined 23(5.2%) were blind leaving 421 quarters functional. Sub clinical mastitis was diagnosed by using California Mastitis Test reagent. Different bio- chemical tests were used to identify bacteria and the highest prevalent bacteria was found to be *Coagulase negative staphylococci (CNS)* (37.7%) followed by *Staphylococcus aureus* (19.6 %), *Escherichia coli* (9.4%), *Staphylococcus intermedius* (9.4%), *Bacillus species* (8%), *Streptococcus species* (5.8%), *Klebsiella pneumoniae* (5.8%), and *Enterobacter aerogens* (4.3%). Among seven anti-biotics tested *in vitro* most isolates were sensitive to Norfloxacin but showed resistance to Ampicillin. Age, stage of lactation, milk yield, hygiene and feet problems and udder conformation were found to be risk factors to mastitis with significant difference $P < 0.05$. However, parity, blind teat and body condition score showed non-significant difference ($P > 0.05$). The high prevalence rate of mastitis in this study implied that, it is the trickiest health problem of dairy cows of the study area. Isolation of both environmental and contagious bacteria revealed unhygienic milking procedure. Therefore, milkers should be trained on proper hygienic milking methods, regular investigation of mastitis especially sub clinical form should be practiced, mastitis treatments should be preceded with identification of the causative agent and susceptibility test profile of pathogens and culling of old aged and repeatedly infected cows should be done on regular planned basis.

Key words: Alage, anti-biotics, bacterial isolates, clinical, dairy, exotic, mastitis, prevalence, risk factors, sub clinical.

1. INTRODUCTION

Javaid *et al.* (2009) implied that general health and welfare of individuals depends largely on meeting basic nutritional needs. Milk and fermented milk products have formed an important part of daily nutrition and the variety of products produced from milk has increased dramatically over the years, as modern food processing technologies have improved. Also, an increase in the global population coupled with the increasing demands for milk as an economic food and as an industrial raw food product has necessitated an increase in production by dairy farms.

Breeding goal in dairying is maximizing profitability by selecting animals with high production traits that remain as much as possible in herd avoiding problems. However, functionality has been endangered through years because of exhaustive selection on increasing production level and antagonistic genetic correlations between production and resistance to some diseases (Rauw *et al.*, 1998). Then, nowadays profitability depends on reducing costs more than increasing income and selection is focusing on functional traits, such as fertility, diseases and calving ease (Philipsson and Lindhe, 2003).

Mastitis is defined as an inflammatory reaction of the mammary gland International Dairy Federation (IDF), (1987). It is induced when pathogenic microorganisms enter the udder through the teat canal, overcome the cow's defense mechanisms, begin to multiply in the udder, and produce toxins that are harmful to the mammary gland. Mammary tissue is then damaged, which causes increased vascular permeability. As a result of this, milk composition is altered: there is leakage of blood constituents, serum proteins, enzymes, and salts into the milk; decreased synthesis of caseins and lactose; and decreased fat quality (Osteras, 2000). The extent of these changes is determined by the severity of the infection (Pyorala, 2003; Harmon, 1994, IDF, 1987).

Oviedo-Boyso *et al.* (2007) and Suriyasathaporn *et al.* (2000) revealed that mastitis is a multifactorial disease. As such, its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms, and presence of environmental risk factors, as well as interactions between these factors. Seegers *et al.* (2003) indicated that mastitis has been described as the most common and costly disease in dairy production causing over 38% economic losses due to health problems. Mastitis frequency increased dramatically in the last decades. Heringstad *et al.* (1999) found that mastitis incidence in 1994 (28%) was two times the frequency in the year 1978. In the last ten years, depending on populations and lactation number, averages ranged from 12% to 40%.

Risk factors associated to clinical mastitis are milking routine, type of housing, feeding, and season, as environmental effects. In addition, older cows, later first calving, first stages of lactation and cows with deep udders, weak attachments, and high production are more liable to mastitis. Health problems have negative consequences not only on animal welfare but also in economics of herds because of additional costs in veterinary, medicines, reduction of production, discarded milk, and involuntary culling (Collard *et al.*, 2000). Thus, economic losses due to mastitis are an important concern for dairy producers though some costs are not obvious (Osteras, 2000).

Mastitis continues to be the most economically important disease of dairy cattle, due to the expense of antibiotic treatment, along with the associated costs of decreased milk production and decreased fertility or, in cases where antibiotic treatment is ineffective, culling or death (Bradley, 2002).

Various researchers revealed mastitis as grievous disease in the dairy industry of different parts of Ethiopia and it has long been known and its prevalence, associated potential risk factors and anti-bio gram susceptibility test was studied in some part of different agro-ecological zone of the country. (Bishi, 1998; Nesru, 1999; Mungube, 2001,

Workneh *et al.*, 2002; Kerro and Tareke, 2003; Mungube *et al.*, 2004; Demelash *et al.*, 2005 Sori *et al.*, 2005; Getahun *et al.*, 2008; Gizat *et al.*, 2008 Matios *et al.*, 2009, Girma, 2010, Mekonnen and Tesfaye, 2010 and Nibret *et al.*, 2011).

Mastitis, known to be a complex and costly disease of dairy cows, that results from the interaction of the cow and environment including milking machine and microorganism (Azmi *et al.*, 2008). Mastitis has been known to cause a great deal of loss or reduction of productivity to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age (Vaarst and Envoldsen, 1997). Moreover, due to its latent form, heavy financial losses and great nutritional and technological impacts can be resulted. Because valuable components of the milk like lactose, fat and casein are decreased while undesirable components like ions and enzymes are increased and making the milk unfit for processing technology (Girma, 2001).

Many infectious agents have been implicated as cause of mastitis in cattle the most common organisms being *Streptococcus agalactiae* and *S. aureus* whereas, environmental mastitis is associated with Coliforms and environmental Streptococci that are frequently found in the cow's environment (Quinn *et al.*, 2002; Radostits *et al.*, 2000).

Mastitis causes a reduced milk production, not only at the occurrence of the mastitis but throughout the rest of the lactation (Hagnestam *et al.*, 2007), increases the risk of new cases of mastitis (Edinger *et al.*, 1999; and increases the risk of culling (Schneider *et al.*, 2007). Schneider *et al.* (2007) revealed that welfare of the cow is negatively influenced by mastitis as it can induce pain and even cause death. Consequences for the farmer are economic losses mainly due to reduced milk production and increased culling. Mastitis is not just an issue for the cow and farmer, but also for the consumers. Consumers expect that milk comes from healthy animals, and the quality of milk is negatively influenced by mastitis.

Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for breeders and consequently influences the national income of the country (McDougall *et al.*, 2009). According to Getahun *et al.* (2008) economic losses are due to loss in milk production, discarding abnormal milk and milk withheld from cows treated with antibiotics, degrading of milk quality and price due to high bacterial or somatic cell count (SCC), costs of drugs, veterinary services and increased labor costs, increased risk of subsequent mastitis, herd replacement, and problems related to antibiotics residues in milk and its products.

According to Sharma *et al.* (2007) mastitis is one of the most significant health problems of dairy herds as it causes physical, chemical and bacteriological changes in the milk of dairy animals resulting in inferior quality and quantity of produced milk with possible public health importance. Therefore, conducting research on its prevalence and incidence will contribute a lot to design appropriate preventive measures and treatment regimen in the specific dairy farm.

A.A.T.V.E.T.C. dairy farms which is located at central rift valley area of Ethiopia is the only source of milk and milk products for the total population of 10,000-15,000 residents in the compound and provides milk products for nearby towns like Ziway, Bulbula, Shashemene and Awassa. Conducting researches on the status of intra mammary infection, that can be considered as bottle neck to the production performance is paramount important. However, the information on the prevalence of the disease, associated risk factors, profiles of major mastitis causing pathogens and anti-bacterial susceptibility profile of causative agents in this dairy farm is almost null and therefore,

The general aim of this study was:

- To determine the prevalence of mastitis and the associated potential risk factors in A.A.T.V.E.T.C. dairy farm, presumed to represent dairy herds with similar management practices and agro-ecological environment of the country.

Specific objectives of the study were:

- ❖ To determine the prevalence of clinical and subclinical mastitis at quarter and cow level in A.A.T.V.E.T.C. dairy cows.
- ❖ To explicate the associated risk factors with the outcome of interest.
- ❖ To isolate and identify the bacterial pathogens which cause both clinical and sub clinical mastitis.
- ❖ To conduct antimicrobial susceptibility profile of isolates.

2. LITERATURE REVIEW

2.1. Definitions and general introduction of mastitis

Mastitis, although an animal welfare problem, is a food safety problem and is the biggest economic problem. Mastitis is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue of the udder (Sharma, 2007). It is also defined as inflammation of mammary gland parenchyma, which is caused by bacteria and its toxins (Sharma *et al.*, 2006). The bacterial contamination of milk from affected cows render it unfit for human consumption and provide a mechanism of spread of diseases like tuberculosis, sore-throat, Q-fever, Brucellosis, Leptospirosis etc. and has zoonotic importance (Sharif *et al.*, 2009). The prevalence of mastitis in cows ranges from 29.34% to 78.54% (Sharma and Rai, 1977; Sharma and Maiti, 2009) and 66%-70.32% in buffaloes (Sharma *et al.*, 2004; Sharma *et al.*, 2007).

According to International Dairy Federation (IDF) (1987) mastitis is defined as an inflammation of the mammary gland. Although it may have a traumatic or toxic etiology it is generally a result of microbiological infection. More than 135 different pathogenic microorganisms have been identified as causative agents of bovine mastitis. Bacteria, fungi and yeasts may all play a role; but of these, bacteria have by far the largest part. Staphylococci, streptococci and members of the Enterobacteriaceae are responsible for the majority of infections (Quinn *et al.*, 1994).

Mastitis can be either clinical or subclinical. Clinical cases give rise to visible symptoms. Mild clinical mastitis (CM) causes flakes or clots in the milk, whereas severe cases are associated with heat, swelling and discoloration of the udder, as well as abnormal secretion. Severe CM can also exhibit systemic reactions, such as fever and loss of

appetite. Mastitis can exist in the absence of visible signs of infection, and is then referred to as subclinical mastitis (SCM). SCM is the most prevalent form of mastitis (Akers, 2002). In practice, whether a case of mastitis is classified as clinical or subclinical often depends on how carefully the cow is observed when diagnosis is made (IDF, 1987).

SCM can be diagnosed by presence of pathogens in bacteriological cultures of milk, but bacteriological sampling is not practically feasible as a routine test. The current standard method of detecting SCM is to measure SCC. Other inflammatory parameters, such as electrical conductivity, lactose, lactate dehydrogenase, acute phase proteins, etc. (Hamann, 2005; Pyorala, 2003), have been proposed as indicators of SCM, and some have the potential of being adapted to in-line use.

The duration of infection further classifies mastitis as acute or chronic manifestations, where a sudden onset defines acute cases and chronic mastitis is characterized by an inflammatory process that lasts for months and results in progressive development of fibrous tissue (IDF, 1987).

2.2. Types of mastitis

2.2.1. Subclinical mastitis

Subclinical mastitis is characterized by changes in milk composition *e.g.* somatic cell count (SCC); leukocytes and epithelial cells), and changes in milk pH and ion concentration, without clinical signs of inflammation (Guidry, 2007).

In the healthy lactating mammary gland, the milk SCC is often $< 100,000$ cells/mL of milk, while the SCC can increase to $> 1,000,000$ cells/ml of milk during subclinical mastitis. The major factor affecting the SCC at the herd and individual level is the presence of intra mammary infections (IMM) (Radostits, 2007).

2.2.2. Clinical mastitis

Clinical mastitis is characterized by visual clots or discolorations of the milk, often in combination with tender and swollen udder, sometimes in combination with fever, loss of appetite *etc.* In Sweden, the most common pathogens isolated at clinical cases of mastitis are *S. aureus*, *E. coli*, *Str. dysgalactiae* and *Str. uberis* (Bengtsson *et al.*, 2005).

2.3. Etiology of mastitis

Although about 20 to 35% of clinical mastitis cases are of unknown etiology (Wellenberg *et al.*, 2002), it is widely accepted that bovine mastitis is mainly bacterial in origin. It can be classified as contagious or environmental. In the former case, it is caused by organisms such as *S. aureus*, *Strep. dysgalactiae* and *Strep. agalactiae*, which are all adapted to survive in the udder, causing subclinical infections. Environmental pathogens like *Strep. uberis* or Enterobacteriaceae like *E. coli* are not well adapted to survive within the udder and, instead, they multiply rapidly following invasion, evoke a swift immune response and are eliminated (Bradley, 2002).

The main etiological agents responsible for mastitis infections can be divided into different groups of organisms depending on the source of the organism involved. These include contagious pathogens, environmental bacteria, opportunistic bacteria and other organisms that less frequently cause mastitis less frequently (Philpot and Nickerson, 1999).

Contagious microorganisms are usually found on the udder or teat surface of infected cows and are the primary source of infection between uninfected and infected udder quarters, usually during milking. The organisms that fit into this category include: *Staphylococcus aureus* (coagulase-positive staphylococci), *Streptococcus agalactiae* and

the less common sources of infection caused by *Corynebacterium bovis* and *Mycoplasma bovis* (Philpot and Nickerson, 1999).

According to Quinn *et al.* (1999) a large number of Gram-positive and Gram-negative species are in a cow's environment and they cause clinical or subclinical infections in the udder and fall into a descriptive category known as environmental mastitis pathogens such as *Streptococcus uberis*, *Streptococcus equinus*, *Enterococcus faecalis* and *Enterococcus faecium* are Gram-positive species. Gram-negative species include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Pseudomonas* spp. Environmental pathogens require moisture, favorable pH and organic material for survival and they enter the gland through the teat canal. Environmental pathogens reside in soil, bedding materials, manure and other organic matter. Therefore, efforts at prevention or control of environmental mastitis should focus on cleanliness of a cow's workplace and cleanliness of a cow. Mastitis caused by environmental organisms is essentially opportunistic in nature and becomes established if the immune system of the host is compromised or if sanitation and hygiene is not adequately practiced (Schukken *et al.*, 2005).

Mekonnen and Tesfaye (2010) revealed that contagious bacteria like *Coagulase negative staphylococci* (CNS), *S. aureus*, *S. agalactiae*, *S. dysgalactiae* and environmental microorganisms like coliforms (*Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus uberis*) were found to be the major etiology of mastitis in market oriented smallholder dairy farms in Adama, Ethiopia. According to Nibret *et al.* (2011) bacterial isolates in the milk of dairy herds in and around Gondar, Ethiopia were *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, CNS, *Micrococcus* species, *Bacillus cereus*, *Corynebacterium bovis* and *Actinomyces pyogenes*.

2.4. Diagnosis of mastitis

2.4.1. Qualitative examination of milk

According to Quinn *et al.* (1994) changes in color of milk can be caused by the presence of blood (red or brownish) or pus (yellow). The consistency may be increased, resulting in thicker, "sticky" milk, or it may be more than usually watery. Flakes and clots are always abnormal. The smell of the secretion may also be altered as a result of mastitis.

2.4.2. California Mastitis Test (CMT)

This practical test was developed in the 1950's during a California testing program; it gives a measure of the SCC of the sampled milk. The reagent (3% sodium lauryl sulphate is often used) is a detergent which ruptures somatic cells in the milk, thereby releasing DNA. This forms a precipitate with other serum components, fat particles and the CMT reagent, causing visible gelling of the milk. A pH-indicator (for example bromcresol purple) may be added to the reagent. The test procedure is simple and straightforward: after the stripping milk is discarded, a few streams of (fore) milk from each quarter are milked into four plastic dishes set on a paddle. The paddle is then tipped nearly vertically to drain excess milk. An equal volume of the reagent is added from a plastic squeeze bottle and the two components mixed by swirling (Quinn *et al.*, 2002).

2.4.3. Flow cytometry (FC)

Flow cytometry (FC) is a method by which physical and chemical characteristics of cells or particles can be measured as they travel in suspension past a sensing point. This method has been developed recently to quantify Somatic Cell Counts in milk, and is particularly good for detecting subclinical mastitis (Tian *et al.*, 2005; Holm *et al.*, 2004).

2.4.4. Culture method

The surest way of diagnosing mastitis is by directly isolating and identifying any pathogenic microorganisms which may be present in the milk. This can be achieved by cultural methods and a number of additional determinative tests. To obtain correct results and avoid contamination and hence bias, it is important to work as securely and as accurately as possible under the circumstances. Similarly, the procedure of routine mastitis testing should be standardized and work protocols instituted (Sears, 1993 and Quinn *et al.*, 1994).

2.5. Treatment regimen for mastitis

2.5.1. Treatment of clinical mastitis in practice

Treatment of mastitis should be targeted towards the causative bacteria whenever possible, but in acute situations, treatment is initiated based on herd data and personal experience. Rapid or on-farm bacteriological diagnosis would facilitate the selection of the most appropriate antimicrobial. Treatment protocols and drug selection for each farm should be made by veterinarians familiar with the farm (Sawant *et al.*, 2005; Wagner and Erskine, 2006).

The use of on-farm written protocols for mastitis treatment can promote judicious use of antimicrobials (Raymond *et al.*, 2006; Passantino, 2007). Therapeutic response of the cows can be monitored using individual somatic cell count data if available, or using the California Mastitis Test, and with bacteriological samples in herds with contagious mastitis. In general, the use of narrow-spectrum antimicrobials is preferable. Prudent use guidelines have been developed which also include antimicrobial treatment of mastitis (Anonymouse, 2003; Passantino, 2007). First choice antimicrobials for treating mastitis caused by streptococci and penicillin-susceptible staphylococci are β -lactam antimicrobials, particularly penicillin G. Broad-spectrum antimicrobials such as third or

fourth generation cephalosporins should not be used as first alternatives for mastitis, as they may increase emergence of broad spectrum β -lactam resistance. Systemic treatment is recommended in clinical mastitis due to *S. aureus* and in severe cases of coliform mastitis, preferably in combination with IMM treatment (Barkema *et al.*, 2006). Too short a duration of standard treatment is probably an important reason for poor cure rates in mastitis therapy. A longer treatment improves cure rates, and duration of treatment should generally be extended in mastitis caused by *S. aureus* and *Streptococcus uberis* (Oliver *et al.*, 2004; Deluyker *et al.*, 2005).

Clinical mastitis should be treated for at least three days; this recommended treatment duration is longer than label treatments in many countries. All mastitis treatment should be evidence based i.e., the efficacy of each product and treatment length should be demonstrated by scientific studies (Cockcroft and Holmes, 2003).

2.5.2. Treatment of Subclinical mastitis

Treating subclinical mastitis with antimicrobials is generally not economical during lactation because of high treatment costs and poor efficacy. In a study with a large number of subclinical mastitis cases (Wilson *et al.*, 1999) the overall bacteriological cure rate for antimicrobial treatment was 75% and that for no treatment 68%. The marginal benefit applied for streptococcal mastitis only; in mastitis due to *Staphylococcus aureus*, antimicrobials were equal to no treatment. Treatment of subclinical mastitis will not affect the incidence of mastitis in the herd unless other preventive measures are taken. Studies on treating cows based on high somatic cell counts have generally shown that no effect on milk production has been achieved (Shephard *et al.*, 2000, Hallen *et al.*, 2008). In herd problems caused by very contagious bacteria such as *S. aureus* or *Streptococcus agalactiae* treatment of subclinical mastitis is advised (Wagner and Erskine, 2006).

2.6. Antibiotic sensitivity test

Prescott and Baggott (1988) indicated that knowledge of the *in vitro* antibiotic susceptibility patterns of identified pathogens is useful for determining the therapeutic strategies most likely to be effective, and in acknowledging the development of drug resistance. However, it has limited predictive value. Pharmacokinetic properties such as drug disposition (absorption, distribution, elimination) and drug toxicity are factors which are difficult to reproduce under laboratory conditions and which are not taken into account during routine antibiotic susceptibility testing. Therefore, the susceptibility patterns as classified in the laboratory often will not agree with *in vivo* susceptibility (Sears *et al.*, 1993).

The agar disc diffusion technique (Kirby Bauer method) is the qualitative method most often performed in laboratories. A fixed quantity of a pure culture of the pathogen is spread on an agar medium (by streaking it out with a sterile swab) and individual paper discs impregnated with a known quantity of an antibiotic are placed on this. The culture is incubated for 18-24 hours at 35°C. The antibiotic diffuses into the agar at a certain rate, while the test organism grows on the agar simultaneously. A critical inhibitory antibiotic concentration is reached at a particular distance from the disc, below which there is no inhibition to bacterial growth. Thus a circular inhibition zone is formed around the disc. The diameter of this zone depends on the concentration and rate of diffusion of the antibiotic, the size of the inoculums, the speed with which the bacteria replicate and their susceptibility to the antibiotic. The diameter of this inhibition zone is measured and compared to given values, on which basis the microorganism is classified as being susceptible, intermediately susceptible or resistant (Erskine *et al.*, 2002).

Strict standardization is necessary so that the factors which influence the diameter of the inhibition zone are controlled to the highest possible degree. A set of criteria has been instituted to achieve this. Firstly, the inoculum must be of a size approximating 10⁵

bacteria /ml; this will result in a dense lawn of bacterial growth. The agar plates must be of specified composition (e.g. Isosensitest agar or Mueller-Hinton agar) and thickness (4mm), as these influence the rate of diffusion of the antibiotic; the concentration of the antibiotics in the disc must be known; and the incubation time and temperature must be uniform (18-24 hours at 35 °C) (Sears *et al.*, 1993). The test method used by most laboratories currently is the Kirby-Bauer disc diffusion test (Walker, 2000) which classifies bacterial isolates as either sensitive or resistant. It has been suggested that the calculation of minimum inhibitory concentrations of antibiotic is a more accurate determination of antibiotic efficacy (Bradley *et al.*, 2002); however this technique is not currently carried out at present. Antibiotic sensitivity testing may be of use at the start of a mastitis investigation to confirm that current therapies are still applicable. Antibiotic sensitivity testing may also be of use to rule out antibiotic resistance as a cause of treatment failure, bearing in mind that there are many causes of apparent treatment failure.

Antimicrobials have been used to treat mastitis for more than fifty years, but consensus about the most efficient, safe, and economical treatment is still lacking. The concept of evidence-based medicine has been introduced to veterinary medicine (Cockcroft and Holmes, 2003) and should apply also to treatment of mastitis. The impact on public health should be taken into account as dairy cows produce milk for consumption (OIE, 2008). Antimicrobial treatment of dairy cows creates residues into milk, and therefore residue avoidance is an important aspect of mastitis treatment (Wagner and Erskine, 2006).

Selecting a substance with a low minimum inhibitory concentration value for the target pathogen is preferable, particularly when the antimicrobial is administered systemically. The antimicrobial should have bactericidal rather than bacteriostatic action, because phagocytosis is impaired in the mammary gland (Kehrli and Harp, 2001).

Antimicrobial susceptibility determined *in vitro* has been considered as a prerequisite for treatment. However, activity *in vitro* does not guarantee efficacy *in vivo* when treating bovine mastitis. Antimicrobial resistance amongst mastitis pathogens has not yet emerged as a clinically relevant issue, but geographical regions may differ in this respect. The biggest problem is the widespread resistance of staphylococci, particularly *Staphylococcus aureus*, to penicillin G (Olsen *et al.*, 2006; Hendriksen *et al.*, 2008). Cure rates for mastitis caused by penicillin-resistant strains of *S. aureus* seem to be inferior to those of mastitis due to penicillin-susceptible strains (Sol *et al.*, 2000 and Taponen *et al.*, 2003). It is not known if this is due to pharmacologic problems of the drugs used, or virulence factors possibly linked to β -lactamase gene of the resistant isolates (Haveri *et al.*, 2005). Using an *in vitro* β -lactamase test for determining resistance to penicillin G of staphylococci before treatment is recommended (Olsen *et al.*, 2006).

Coagulase negative staphylococci tend to be more resistant than *S. aureus* and easily develop multi resistance (Sawant *et al.*, 2009). Mastitis causing *streptococci* have remained susceptible to penicillin G, but emerging resistance to macrolides and lincosamides has been detected (Loch *et al.*, 2005).

2.7. Basic facts for control of mastitis

Philpot and Nickerson, (1999) indicated that milking practice is of paramount importance as this is common route of infection. The udder should be prepared before milking by washing the teats, followed by disinfection and drying with clean paper towels. If the teat area is dripping with water from run-off of areas that were heavily soiled it could lead to pathogens gaining access to the teat canal. Milker's hands should also be disinfected to prevent the transfer of pathogens. Post milking treatment is also important and all cows should be treated with a teat dip disinfectant to reduce the risk of infection. A strategy to control mastitis must be practical and economical. The primary goal would be to reduce the rate of new infections and the duration of current

infections within a herd. It would also be essentially important to maintain normal udder health ensuring that the natural immune response in the cow can resist and fight disease while still producing the required level of milk.

Control strategies need to target every facet and process of dairy farming and can begin with maintaining good hygiene practices in the environment. The holding yards or stalls should be kept clean and dry. The water supply should be adequate and free of coliform bacteria and equipment should be maintained and sanitized between milking. The welfare of animals is becoming increasingly important in modern dairy production as consumers become more concerned about the manner in which farm animals are treated. The Farm Animal Welfare Council in the UK has defined “the five freedoms” of animals, which highlight issues relating to the treatment and management of animals. The advantage of implementing such quality control measures within the herd would ensure that dairy cows are free of a stressful environment, injury, pain, hunger and discomfort, which in turn would promote a healthy immune system and udder health in general (Sandgren and Ekman, 2005).

The control of mastitis has been successfully achieved through the establishment of effective herd health control programs (Erskine *et al.*, 2002). Antimicrobial agents are the main therapeutic tools for the treatment and control of mastitis. Among main reasons of low efficacy of antibiotic treatment of mastitis cases is the resistance of the bacteria to antimicrobials. (De Olivera *et al.*, 2000, Gitau *et al.*, 2003 and Haile, 2004).

2.8. Prevalence of mastitis

Mungube (2005) revealed overall prevalence of sub clinical mastitis in cross breed dairy cows in Ethiopia was 52.3% and 32.4% at cow and quarter level respectively. According to Regassa *et al.* (2010b) in dairy farms of Holeta town, central Ethiopia prevalence of mastitis at cow level was found to be 71.0% (76/107), out of which 22.4% (24/107) and

48.6 % (52/107) were clinical and subclinical, respectively. The quarter level prevalence was 44.9% (192/428); from this the clinical form was 10.0% (43/428) and the subclinical was 34.8% (149/428). Out of the 43 quarters clinical cases, 31 had blind teats while 12 of them revealed active cases of mastitis showing visible sign of inflammation on the udder and changes were also observed on milk.

According to Regassa *et al.* (2010a) research conducted in Adama town, Ethiopia among 300 lactating cows examined, 140 (46.7%) had mastitis, of which 10.0% (30/300) and 36.7% (110/300) showed clinical and subclinical mastitis, respectively. The quarter level prevalence was 29.0% (348/1200); from which 23.5% (283/1200) and 1.5% (18/1200) were found to be of subclinical form and blind teat, respectively. The remaining 3.9% (47/1200) were of a clinical form revealing active cases of mastitis with visible signs of inflammation on the udder and changes in milk quality.

Getahun *et al.* (2008) indicated that in Selalle smallholder dairy farms, central Ethiopia, a total of 14 (12.8%) herds, 16 (3.2%) cows and 17 (0.9%) quarters had clinical mastitis while 77 (70.6%) herds, 147 (30.4%) cows and 264 (13.6%) quarters had sub-clinical mastitis.

According to Mekonnen and Tesfaye (2010) in smallholder dairy farms of Adama, Ethiopia 62%, 48% and 24.6% at herds, cows and quarter level respectively were affected by clinical and/or sub-clinical mastitis. Out of the total number of 195 quarters affected by clinical and sub-clinical mastitis 48(24.6%) were right fore, 50(25.6%) right hind, 53(27.2%) left fore and 44 (22.6%) left hind quarters. The prevalence of sub-clinical mastitis was higher than clinical mastitis at herd, cow and quarter levels, respectively, by 4.5, 6.6 and 9.3 times.

2.9. Mastitis and its potential associated risk factors

2.9.1. Parity

(Demelash *et al.* 2005, Gizat *et al.* 2008, Rahman *et al.* 2009, Matios *et al.* 2009 and Molalegn *et al.* 2010) indicated that the higher the parity numbers the more the prevalence of mastitis. According to Steeneveld *et al.* (2008) in multiparous cows, the risk of developing clinical mastitis (CM) increases with increasing parity. In Sweden, most first-parity cows calve and start to lactate at about 2-2.5 years of age. To pay for the costs of rearing it is very important that the first-parity cow is healthy, and able to produce good quality milk, which also will enhance longevity. Unfortunately, first-parity cows have been shown to have as high, or higher, incidence of udder disorders in early lactation as older cows (Valde *et al.*, 2004). This can be detrimental to her future life due to reduced milk production (Hagnestam *et al.*, 2007), increased risk of new cases of mastitis (Edinger *et al.*, 1999) and increased risk of culling (Schneider *et al.*, 2007). According to Skrzypek *et al.* (2004) the level of SCC has been reported to be influenced by parity and SCC increases with advanced parities.

2.9.2. Udder conformation and prevalence of mastitis

Each quarter is composed of the milk-producing tissue or alveoli that lead into the lactiferous ducts, gland cistern, teat canal and finally the teat opening or duct. The alveoli are lined with epithelial cells that become specialized during the gestation period, before calving, and after calving. These specialized cells produce colostral and lacteal secretions and finally, milk. Connective tissue and muscle cells support the alveoli glands and contract and squeeze milk from the alveoli during milking (Philpot and Nickerson, 1999).

To treat mastitis infections effectively, it is important to understand the invasive patterns of the different pathogenic bacteria and the host immune response to these pathogens.

Mastitis occurs when microorganisms enter via the teat opening or duct and are able to overcome the immune system, multiply and establish within the teat canal and the mammary tissue. Invasion of the udder most likely occurs between milking periods. This is when microorganisms are present on the outer surface of the udder, on milking machines, or on the hands of workers. The opening of the teat canal has sphincter muscles that provide a physical barrier from the outside and is able to maintain a tight closure of the opening (Philpot and Nickerson, 1999). In addition, the teat canal is also lined with keratin which is a waxy substance derived from squamous epithelial cells. The keratin not only acts as a barrier between invading organisms and the gland cistern, but also contains bacteriostatic anti- microbial agents (Sordillo and Streicher, 2002). This physical barrier can be compromised through trauma incurred, or microorganisms can simply be propelled through the teat canal during the use of milking machines (Philpot and Nickerson, 1999). These anatomical factors are the first line of defense against colonization and form part of the innate or non-specific immune response in the mammary gland (Oviedo-Boyso *et al.*, 2007).

According to Girma (2010) and Sori *et al.* (2005) animals with pendulous udder showed higher incidence of mastitis than cows with non-pendulous udder and there was an association between the two categories this is because of more exposure to the environmental pathogens and injurious materials.

2.9.3. Stages of Lactation

The prevalence of mastitis was significantly higher at 6-10 months after calving than 1-5 months after calving (Rahman *et al.*, 2004). The highest prevalence of sub clinical mastitis occurred during the 4th months of lactation while the lowest during 5th or more than 5th months of lactation (Rahman *et al.*, 1997). Gizat *et al.* (2008) revealed stage of lactation was found to be significant with the occurrence of mastitis.

Risk of new environmental streptococcal infection is influenced by stage of lactation, parity, nutrition, and immunity in addition to factors that increase teat end exposure. The importance of the dry period in control of environmental streptococcal IMI cannot be over emphasized (Green *et al.*, 2002).

2.9.4. Age of cows

The study conducted in different part of Ethiopia by different authors, (Mungube *et al.* 2004, Demelash *et al.* 2005 and Regassa *et al.* 2010b) indicated that age considered as potential risk factor to the prevalence of mastitis. As the age of cow advances the prevalence rate become higher (older cows were more affected by mastitis than younger cows), with prominent statistical variation ($p < 0.05$).

2.9.5. Body condition score of cows

High-yielding dairy cows usually exhibit a negative energy balance after calving, which may influence both the immune system and the metabolic system of the individual (Goff and Horst, 1997). Chronic deficiencies of energy, protein, minerals, or vitamins have repeatedly been associated with increased disease susceptibility as a result of depressed immune function. Because most udder infections occur in the peri partum period, optimal feeding, both in the dry period and during early lactation, may be important in preventing mastitis (Gearhart *et al.*, 1990). It seems difficult for the high-producing dairy cow to ingest enough feed shortly after calving to meet lactation demands for energy and protein. Cows with high BCS at calving lose more condition and achieve positive energy balance later than do cows with lower BCS (Ruegg and Milton, 1995). These findings suggest that there might be differences in the length and severity of the immunosuppressive period after calving, which may influence the risk for mastitis.

Most studies of the association between BCS and post parturient diseases have been based on experiments involving a limited number of individuals. The significant

difference in mean BCS between high- and low-infection herds around calving showed that cows in the high-infection herds had higher BCS and thus may have experienced a larger drop in BCS during the first 2 months of lactation (Valde *et al.*, 2007). Having a high proportion of cows in the herd with intermediate BCS was found to be a risk factor for the development of toxic mastitis (Tadich *et al.*, 1998). According to Mekonnen and Tesfaye (2010) a cow with poor body condition score and with previous exposure to mastitis were more liable to mastitis than a cow with good body condition score and non-exposed once respectively in the study conducted on dairies of Adama, Ethiopia.

2.9.6. Milk yield

A high 305-day previous-lactation milk yield was a significant risk factor for early lactation clinical mastitis and high yields increased the mastitis rate in low bulk milk somatic cell count herds (Schukken *et al.*, 2005). A high milk protein content at the last milk-test day prior to drying-off was been found to be a risk factor for early lactation CM. This may reflect higher energy supplies to the udder and lead to delayed involution of the udder tissue. Cows with a fat to protein ratio of >1.5 at the first test-day after calving had higher risks for clinical mastitis and other production diseases in a study by (Heuer *et al.*, 1999).

2.9.7. Hygiene scoring

The environment in which dairy cows are kept has a decisive effect on their health and welfare. A clean and comfortable shelter represents the key to maintaining the dairy cows' health and longevity. The shelter's hygiene level can be evaluated through several assessment systems based on the quantification of the manure pollution in different body regions of the cows (Chaplin *et al.*, 2000). It is stated that the majority of these systems failed as practical hygiene monitoring tools at farm level, apart from their value in scientific research (Cook, 2002). For a scoring system to be useful both for veterinarians and farmers, the significance of manure contamination in different body areas must be

understood and the pollution level compared to an established standard, derived either from the contamination level of the same farm in time, or from the data obtained in some similar farms (Cook, 2002). For the hygiene scoring to be taken seriously, the farmer must understand what the costs of keeping animals in a dirty environment are. For dairy cows the outcomes of low hygiene are the high risk of mastitis and the worsening of lameness. The relation between shelter hygiene, clean cows and low number of somatic cells in mixed milk were indicated in several studies (Barkema *et al.*, 1999; Barkema and Schukken, 2003;).

The hygiene scoring system was elaborated by Cook (2002) in order to quantify the hygiene level in the farm and for the assessment of the improvements which have to be made in hygiene management. This system is considered a remedial tool of the existing deficiencies.

Schreiner (2003) indicated that manure on teat ends is an environmental hazard (determinant) for the cause of mastitis when bacteria in manure enter the mammary gland. A cow's chances of mastitis increase with the number of her teats covered with manure (all four teat ends vs. one teat end), the frequency of contamination (negligible versus every day) and duration of time (negligible versus most of the day). For a cow lying in slurry, the environmental hazard is obviously manure.

At the herd level, choices in bedding type, slurry removal from alleys or housing type may be global hazards that affect the frequency of environmental mastitis. Risk is a measure of the likelihood of occurrence and the magnitude of the consequences of an adverse outcome such as mastitis. In veterinary medicine, there is scant information to quantify the risk of environmental mastitis. Researchers report incidence, prevalence, associations or likelihood ratios but seldom quantify impact. For example, a researcher may report cows with dirty udders are 1.5 times more likely to have major pathogens isolated from their milk samples than cows with cleaner udders (Schreiner, 2003). Others

report a positive association between dirty udders and hind limbs and individual cow somatic cell counts (Reneau *et al*, 2005). According to Molalegn *et al*. (2010) and Matios *et al*. (2009) the cow's hygiene significantly affects the prevalence of mastitis.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted at Federal Ministry of Agriculture A.T.V.E.T.C. from October 2011 to May 2012 which is located at longitude of about 38°30' East and latitude of 07°30' North with a total area of 4200 hectares with an altitude of 1600 meters above sea level. The area is situated at 217 kilometers southwest of Addis Ababa and 32 km west of Bulbla town along Addis Ababa-Awasa highway road. Agro-ecologically it is dry plateau of the southwestern part of the Ethiopian central rift valleys. The area has three distinct seasons, namely main rainy (June to September), short rainy (March to May), and dry (October to February)., the mean annual rainfall of the area is 800 mm, with minimum and maximum temperatures of 11 and 29 °C, respectively based on data (1996-2011).

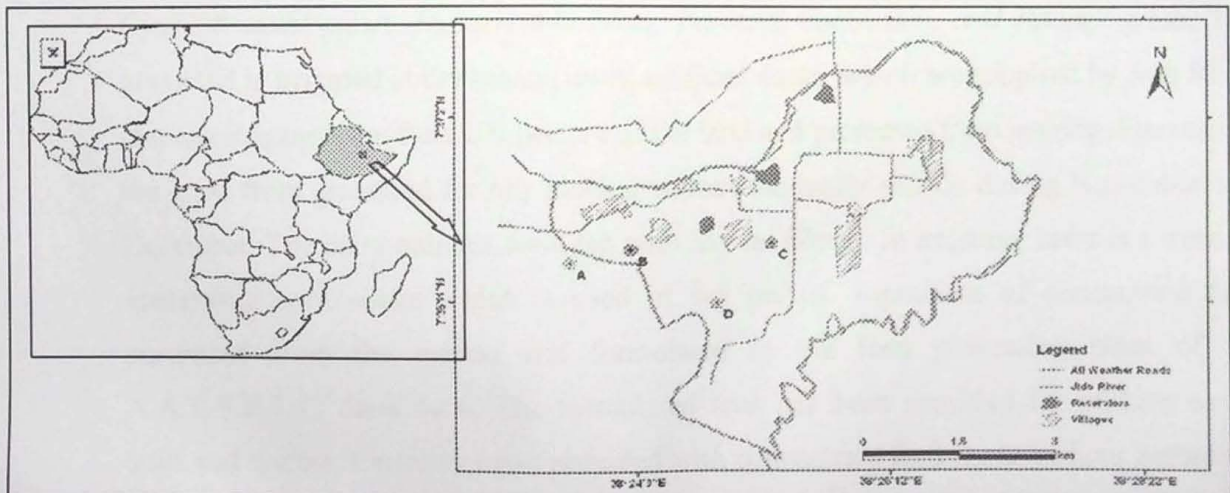


Figure 1 Map of Alage area and location of study sites (A= wheat farm, B= bushland, C= *Acacia* woodland, D= maize farm) (Addisu, A. 2007).

3.2. Study Population

All dairy Holstein Friesian cattle present at A.A.T.V.E.T.C. constituted the study population. Currently there are 205 Boran and 200 heads of Holstein Friesian cattle, 300 heads of pigs, 5000 chickens, 15 camels, 120 sheep and goats at the College farm.

The A.A.T.V.E.T.C. dairy farm was established in 1983 by introducing 300 Holstein Friesian heifers from Stella, Holetta, and private dairy farms around Addis Ababa. At the time of establishment, the primary objective of the dairy farm was to provide milk and milk by-products to orphan children, as the institute itself was an orphanage. Dairy animals are kept at in door; feeding and the management system of the farm can be considered as intensive.

Grass was usually harvested from the naturally conserved open savannah grassland and conserved as hay by bailing system. The dominate grass species are *Cenchrus ciliaris*, *Cynodon aethiopicus*, *Hyparrhenia hirta*, *Panicum coloratum*, and *Rhodes* grass. The grassland is irrigated at dry season using artificial dams, which are supplied by Jido River that can irrigate more than 200 hectare of the land and protected from grazing. Harvesting the grass from grassland for hay making is done manually mainly during November and December. The dairy animals were fed with hay *ad libitum*. In addition, there is a trend of conserving corn silage which is used in dry period. Ingredient of concentrate feed purchased from the market and formulated in the feed processing plant of the A.A.T.V.E.T.C. dairy farm. The formulated feed has been provided for milking cows, bulls and heifers. Lactating cows provided with concentrate feed about an hour before the time of milking. In addition, milking cows have been allowed to feed on green fodders harvested from forage site. Calves have been separated from their dam immediately after birth and colostrum was provided through bucket. Cows have been milked by hand twice a day, morning and evening at (5:00 AM and 3:00 PM). The volume of milk of each cow is measured immediately and recorded.

The dairy farm has been using pure Holstein Friesian and Boran- Holstein cross semen from National Artificial Insemination center for inseminating cows. The insemination is carried out by an AI technician. Heat detection is usually done from 6:00 AM to 9:00 AM in exercise pen.

Veterinary services are carried out and the animals are regularly vaccinated against all viral and bacterial diseases which are prevalent to the area. External and internal parasite control program is done at regular basis, clinically visible disease are treated immediately. There are also regular tests of Brucellosis and Tuberculosis and animals have been culled based on the result. However, there is no practice of detecting sub clinical form of mastitis in the farm except handling mastitis with clear cardinal signs.

3.3. Study design and sample size

A cross-sectional type of study was carried out from October 2011 to May 2012 to investigate the prevalence of clinical and sub-clinical mastitis at cow and quarter levels and to detect possible association between various risk factors and isolation of etiological agents on 444 quarters of 111 lactating cows.

3.4. Study Methodology

The study methodology involved reviewing farm documents, farm inspection, animal examination and laboratory investigation. Farm records with respect to animals' parity, stage of lactation, past disease history, production performance were reviewed. Relevant information related to the previous health history of the mammary quarters was obtained from case record book. Other available farm documents were also read.

3.4.1. Farm inspection

Farm inspection was practiced to assess the housing conditions, feeding practices and milking practices. The housing condition was qualified as poor when there is bad smell, feed trough and gutter (for waste drainage) were dirty, animals flank, udder and belly were soiled. The housing condition was qualified as good when none of the above indicated defects were observed. Milking practice was investigated through close observation at the time of milking.

3.4.2. Animal Examination

Animal examination was conducted to determine their body condition, presence or absence of feet and leg problems, soundness of udder and hygiene score. Body condition scoring was implemented using 1-5 point scale based on palpation of back bone and lumbar process and evaluation of coverage of fat and muscle. Presence or absences of feet and legs problems were evaluated through visual inspection and palpation. Hygiene scoring of cows was determined based on a scale of 1 - 4 for three zones of the body; udder, lower leg and upper leg and flank (Chaplin *et al.*, 2000).

Morphology and udder attachment was first examined visually and then through palpation for possible fibrosis, cardinal signs of inflammation, visible injury, atrophy of the tissue and swelling of the supra-mammary lymph nodes were identified. The size and consistency of mammary quarters was inspected for abnormalities, such as asymmetry, swelling, firmness, and blindness. Physical appearance of milk secretion from each mammary quarter was examined for the presence of clots, flakes, blood and watery secretions. Presence of hotness, redness and painful sensation was detected by inspection and palpation.

3.4.3. Preparation of udder and teats for milk sample collection

The udder, especially the teats were cleaned and dried before milk sample collection. Dust, particles or other filth was removed by brushing the surface of the teats and udder with a dry towel. The teats were washed with tap water and dried. Then the teats were disinfected with cotton soaked in 70% ethyl alcohol.

3.4.4. Sampling method and Sample handling

Milk samples were collected by standard milk sampling techniques from all lactating cows with clinical and sub clinical mastitis. To reduce contamination of the teat ends during sample collection, the near teats were sampled first followed by the far once. Approximately 10 ml of paired milk was collected from each quarter (one for CMT and one for bacteriological examination) into labeled sterile screwed cap universal bottle after discarding the first three milking streams. Samples were placed in ice box and transported to the Federal Ministry of Agriculture, A.A.T.V.E.T.C. Microbiology laboratory and processed as soon as possible without any delay.

3.4.5. California Mastitis Test (CMT)

Subclinical mastitis cases were diagnosed based on CMT results and the nature of gel formation (milk and CMT reagent), which shows the presence and severity of the infection. Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT according to Quinn *et al.*, (1999). From each quarter of the udder, a squirt of milk sample was dropped in each of the strip cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed gently. The test result was interpreted based on the thickness of gel formed by CMT reagent and milk mixture and scored as 0(negative), T (trace), 1(weak positive), 2(distinct positive) and 3(strong positive).

Finally quarters with CMT score of 1 or above were judged as positive for sub clinical mastitis; otherwise negative (Quinn *et al.*, 1999).

3.4.6. Bacteriological isolation and identification

Milk samples from both clinical and sub clinical quarters were bacteriologically examined according to the procedures employed by (Quinn *et al.* 1999). Before inoculating into primary culture medium, the milk samples were centrifuged so as to increase the bacterial load. A loop full of centrifuged mastitic milk sample was taken from each infected quarter and inoculated separately on to blood agar base enriched with 7% ovine blood using quadrant streaking method. The inoculated plates were incubated aerobically at 37°C for 24 to 48 hours, after which presence or absence of bacterial growth, colony morphology, color and hemolytic characteristics were recorded on primary culture.

Prior to further biochemical tests, the isolated bacteria on blood agar were sub cultured into nutrient agar. Each culture was subjected to gram staining to determine their shape, and gram reaction. Catalase test using 3% Hydrogen per oxide (H₂O₂) was performed to identify catalase positive and catalase negative bacteria. Mannitol Salt Agar (Oxiod,UK) and purple base agar (Difco) with 1% maltose were used to differentiate staphylococcus species and incubated at 37°C and examined after 24–48 hrs. for mannitol and maltose fermentation respectively. Tube coagulase test using rabbit plasma was used to identify the coagulase positive and coagulase negative staphylococcus species. Enterobacteriaceae species were identified using Oxidase test, SIM medium (Oxiod,UK) for sulfur production, indole test after addition few drops of kovacs reagent and motility test, TSI (Triple Sugar Iron) (Oxiod,UK) to detect sugar fermentation, sulfur and gas production, MacConkey agar (Oxiod,UK) for lactose fermentation and colony characteristics and Simmon's citrate agar (Oxiod,UK) to differentiate bacteria based on citrate utilization .

3.4.7. Antibacterial sensitivity test

Kiby-Bauer disk diffusion method was employed to test *in vitro* antibiotic sensitivity test (Quinn *et al.*, 1994). After identifying isolated colonies through different biochemical tests, each isolates was suspended in to Tryptose Soya Broth (TSB) (oxiod, UK) then incubated for 24 hours. Finally, bacteria suspended in TSB media were spread in to Mulluer Hinton agar and blood agar (oxiod, UK) using cotton swab. Seven different antibiotic discs like Norfloxacin, Ampicillin, Gentamicin, Doxycycline, Erythromycin, Trimethoprim- Sulfamethoxazole and Tetracycline were chosen, because these drugs were used in the study area for treatment of different diseases. Finally, they are dispensed on the medium using forceps and incubated for 24 hrs. Diameter of zone of inhibition for each anti-biotic disc was measured using a ruler in to the nearest millimeter and interpreted as resistant and sensitive according to the standard given by Quinn *et al.* (1994) and manufacturer (Oxoid) instruction.

3.5. Data entry and Analysis

Data were coded, cleaned and entered into Microsoft Excel computer software. Statistical analysis was carried out using SPSS version 20. Data were analyzed descriptively using descriptive statistics in the first step; thereafter association of the different variables with interest of outcome was analyzed using a Chi-squared (χ^2) test. The association was considered significant when odds ratio was greater than one and p-value was less than 0.05.

4. RESULTS

4.1. Cow data

There were a total of 111 milking cows during the time of the present study at A.T.V.E.T.C. dairy farm. Table 1 illustrates some of the physical and productive characteristics of dairy cows. The age of cows ranged from three to more than eight years with highest proportion (54%) being aged between 3 and 5 years. The majority (58.6%) of the cows were in their first and second lactations. More than 82% of the cows had body condition score of 3-4. Milk yield per day varied from 4 liters to more than 11 liters with 53 % of the cows producing 8-11 liters per day.

4.2. Prevalence of mastitis

In the present study a total of 444 quarter milk samples were investigated from 111 Holstein Friesian lactating cows of A.T.V.E.T.C. large scale state dairy farm. The overall prevalence of mastitis at cow level was found to be 73% with sub clinical and clinical prevalence of 56.8% and 16.2% respectively. The current investigation revealed that the overall prevalence of clinical and sub clinical mastitis at quarter level was 8.8% and 28.2% respectively. Prevalence at right quarters and left quarters was also found to be 54.5 % and 44.5% respectively. The details of prevalence rates of clinical and sub-clinical mastitis at cow and quarter levels are presented in Table 2 and 3 respectively. Table 4 shows that Among 444 quarters examined 23(5.2%) were blind quarters leaving 421(94.8%) quarters functional.

Table .1 Physical and productive characteristics of milking cows at Alage dairy farm

Category	Variable	Frequency	Percent
Age	Young (3-5 years)	60	54.05
	Mid age (6-8 years)	18	16.22
	Old age (>8 years)	33	27.73
Parity	Few (1-2 lactations)	65	58.56
	Many (≥ 3 lactations)	46	41.44
Stages of lactation	Early (1-3 months)	41	36.94
	Medium (4-6 months)	30	27.03
	Late (>6 months)	40	36.04
Average daily milk yield	High (> 11 liter)	35	31.53
	Medium (8-11 liter)	59	53.15
	Low (4-7 liter)	17	15.32
BCS (on a scale of 1-5)	Good (3-4)	92	82.88
	Poor (1-2)	19	17.12
Feet problems	Yes	43	38.73
	No	68	61.26
Udder conformation	High up	45	40.54
	Pendulous	66	59.46
Blind teat	Yes	23	20.72
	No	88	79.28
PETM	Yes	54	48.65
	No	57	51.35
Hygiene score (On a scale of 1-4)	Good (1-2)	65	58.56
	Bad (3-4)	46	41.44

PETM = previous exposure to mastitis

Table 2. Cow level mastitis prevalence (n=111).

Types of mastitis	Total number of positive	Prevalence rate
Clinical	18	16.2%
Sub clinical	63	56.8%
Total	81	73%

Table 3. Quarter level mastitis prevalence (n=421).

Types of mastitis	Quarter level prevalence				Total
	Right front	Right hind	Left front	Left hind	
Clinical	11(2.6%)	8(2%)	7(1.7%)	11(2.6%)	37(8.8%)
Sub clinical	30(7.1%)	36(8.6%)	26(6.2%)	27(6.4%)	119(28.2%)
Total	41(9.7%)	44(10.5%)	33(7.8%)	38(9%)	156(37%)

Table 4. Quarter level blind teats distribution (n=444).

Quarters	Total No examined	No of blinded teats	Proportion
Right fore	111	4	3.6
Right hind	111	4	3.6
Left fore	111	7	6.3
Left hind	111	8	7.2
Total	444	23	5.2

4.3. Bacteriological examination result

From a total of 156 quarter milk samples (37 clinical and 119 sub-clinical) cultured 138 were culture positive. Ten (6.4%) were rejected for contamination and 8 (5.1%) yield no bacterial growth. All the clinical mastitis and 101 of the sub-mastitis samples resulted in positive culture. Contagious pathogens like *Staphylococcus* bacterial species and environmental pathogens like *Escherichia.coli*, *Klebsiella pneumoniae* and *Enterobacter aerogens* were identified. The highest prevalent bacteria was found to be *Coagulase negative staphylococci (CNS)* (37.7%) followed by *Staphylococcus aureus* (19.6 %), *Escherichia coli* (9.4%), *Staphylococcus intermedius* (9.4%), *Bacillus species* (8%), *Streptococcus species* (5.8%), *Klebsiella pneumoniae* (5.8%), and *Enterobacter aerogens* (4.3%) in that order. The detail of bacterial isolates (Table 5).

Table 5.Types and frequencies of bacterial isolates with status of mastitis.

Types of bacteria isolated	Status of mastitis		Total frequencies	proportion
	Clinical mastitis	Sub clinical mastitis		
*CNS	15(10.9%)	37(26.8%)	52	37.7%
<i>Staphylococcus aureus</i>	5(3.6%)	22(16%)	27	19.6%
<i>Escherichia coli</i>	2(1.4%)	11(8%)	13	9.4%
<i>Staphylococcus intermedius</i>	2(1.4%)	11(8%)	13	9.4%
<i>Bacillus species</i>	4(2.9%)	7(5.1%)	11	8%
<i>Streptococcus species</i>	2(1.4%)	6(4.4%)	8	5.8%
<i>Klebsiella pneumoniae</i>	4(2.9%)	4(2.9%)	8	5.8%
<i>Enterobacter aerogens</i>	3(2.174%)	3(2.174%)	6	4.3%
Total	37(26.8%)	101(73.2%)	138	100%

*CNS (coagulase negative staphylococci)

4.4. Animal and/or management factors associated with mastitis problems

Table 6 illustrates the prevalence of mastitis in relation to age at cow level and it was higher as the age advances with the prevalence rate of 88%, 77% and 63% in older, mid age and young cows respectively. There was statistically significant difference among different age groups ($p < 0.05$).

The current investigation showed that cows with early lactation stage had highest mastitis prevalence (100%) than that of late (68%) and mid (43.3%) lactations with highly significant difference within different stages of lactation $p < 0.01$ (Table 7).

There was also highly statistical difference ($p < 0.01$) between cows with feet problems and without feet problems with prevalence rate of 93% and 60% respectively (Table 8).

Table 6. Cow's age as potential risk factor to mastitis.

Risk factor	Status of mastitis				χ^2	df	P-value
	Total No examined	Clinical (%)	Sub- clinical (%)	Over all (%)			
Age					6.728	2	0.000**
Young	60	11(18.3)	27(45)	38(63.3)			
Mid age	18	0 (0)	14(77.8)	14(77)			
Old age	33	7(21.2)	22(66.7)	29(87.8)			
Total	111	18(16)	63(57)	81(73)			

age in years 3-5 (young) 6-8(mid-age) and (>8) (old age). ** highly significance difference

Table 7. Stages of lactation considered as risk factor to mastitis.

Risk factor	Status of mastitis				χ^2	df	P-value
	Total No examined	Clinical (%)	Sub- clinical (%)	Over all (%)	16.14	2	0.000**
Stages of lactation							
Early	41	9(22)	32(78)	41(100)			
Medium	30	3(10)	10(33.3)	13(43.3)			
Late	40	6(15)	21(52.5)	27(67.5)			
Total	111	18(16)	63(57)	81(73)			

stages of lactation in months 1-3(early), 4-6 (mid) and >6 (late). **highly significance difference.

Table 8. Association of presence of feet problems with mastitis.

Risk factor	Status of mastitis				χ^2	df	P-value	OR	95% CI
	Total No examined	Clinical (%)	Sub- clinical (%)	Over all (%)	12.83	1	0.000**	5.5	2.05-14.68
Feet problems									
Yes	43	8(19)	32(74)	40(93)					
No	68	10(15)	31(46)	41(60)					
Total	111	18(16)	63(57)	81(73)					

** highly significance difference

Table 9 indicates cows with pendulous udder conformation have higher prevalence rate (89%) than high up udder (49%) and there was significant statistical difference ($P < 0.01$) between the two forms of udder conformation. Animals with poor body condition score, multiple parities, and blind teats exhibited higher prevalence rate of mastitis (79%, 80% and 91%) respectively. In contrast animals with good body condition score, few parities and absence of blind teats showed lower prevalence rate of mastitis (72%, 68% and 68%) respectively. Body condition score, parity, and presence of blind teat were however not significantly associated with mastitis ($p > 0.05$). The details of different associated risk factors to prevalence of mastitis are illustrated in the table 10, 11 and 12 respectively.

Table 9. Cow's udder conformation as risk factor to mastitis.

Risk factor	Status of mastitis				χ^2	df	P-value	OR	95% CI
	Total No examined	Clinical (%)	Sub- clinical (%)	Over all (%)					
Udder conformation					22.32	1	0.000**	7.5	3.11-18.0
High up	45	6(13)	16(36)	22(49)					
Pendulous	66	12(18)	47(71)	59(89)					
Total	111	18(16)	63(57)	81(73)					

Table 10. Body condition score of cows as risk factor to mastitis.

Risk factor	Status of mastitis				χ^2	df	P-Value	OR	95% CI
	Total No examined	Clinical (%)	Sub-clinical (%)	Over all (%)					
BCS					0.79	1	0.32	3.4	1.06-11.15
Good	92	18(20)	48(52)	66(72)					
Poor	19	0(0)	15(79)	15(79)					
Total	111	18(16)	63(57)	81(73)					

BSC=body condition score in 1-5 scale; 1-2(poor) and (3-4) good).

Table 11. Associations between parity number and prevalence of mastitis.

Risk factor	Status of mastitis				χ^2	df	P-Value	OR	95% CI
	Total No examined	Clinical (%)	Sub-clinical (%)	Over all (%)					
parity					2.3		0.1218	1.89	0.828-4.307
Few	65	11(17)	33(51)	44(68)					
Many	46	7(15)	30(65)	37(80)					
Total	111	18(16)	63(57)	81(73)					

number of parity few= (1-2) and many (>2), χ^2 (chi-square), df (degree of freedom)

Table 12. Presence of blind teats as risk factor to the mastitis.

Risk factor	Status of mastitis				χ^2	df	P-value	OR	95%
	No of cows examined	Clinical (%)	Sub-clinical (%)	Over all (%)	2.012	1	0.156	2.2	0.74 5.32
Blind teat									
Yes	23	5(21.7)	16(69.5)	21(91)					
No	88	13(14.7)	47(53.4)	60(68)					
Total	111	18(16)	63(57)	81(73)					

Cows with previous exposure to mammary gland infection had more prevalent rate of mastitis than non-exposed ones 89% and 58% respectively (Table 13). Milk yield status was found to a risk factor to mastitis with the prevalence rate of 92%, 71% and 42% in high, medium and low milk producers (Table14). Table 15 describes that hygiene score also found to be one of animal management factor that influenced the prevalence of mastitis in the study period. Cows with bad hygiene score accounted higher rate (100%) than the cows considered as clean (54%).

Table 13 .Association between previous exposure to mastitis and its occurrence.

Risk factor	Status of mastitis				χ^2	df	P-value	OR	95% C
	Total No			Over all					
PETM	examined	Clinical (%)	Sub-clinical (%)	(%)	8.98	1	0.003**	3.52	1.516-8.165
Yes	54	10(19)	38(70)	48(89)					
No	57	8(14)	25(44)	33(58)					
Total	111	18(16)	63(57)	81(73)					

PETM (previous exposure to mastitis) and ** (highly significance difference)

Table 14. Association between prevalence of mastitis and milk yield performance.

Risk factor		Status of mastitis			χ^2	df	P- value
Average daily	No of cows	Clinical	Sub-clinical (%)	Over all			
milk yield	examined	(%)		(%)			
High	35	6(17)	26(74)	32(92)	13.44	2	0.001
Medium	59	10(74)	32(54)	42(71)			
Low	17	2(12)	5(29)	7(42)			
Total	111	18(16)	63(57)	81(73)			

milk yield in liter 4-7(low), 8-11(medium), >11(high), χ^2 (chi-square), df (degree of freedom).

Table 15. Association of cow's hygiene and prevalence of mastitis.

Risk factor	Status of mastitis				χ^2	df	P- value	95%(CI)	Odds ratio
	No of cows examined	Clinical (%)	Sub-clinical (%)	Overall (%)					
Hygiene score					19.05	1	0.000	0.041-0.337	0.118
Good	65	6(9)	29(45)	35(54)					
Bad	46	12(26)	34(74)	46(100)					
Total	111	18(16)	63(57)	81(73)					

hygiene score in 1-4 scales 1-2 (good) and 3-4(bad)

4.5. Antimicrobial susceptibility profile of mastitis isolates

Table 16 shows anti-bio gram test for 138 bacterial isolates was performed through a panel of seven antimicrobial drugs. Anti-biotic discs (Oxoid, UK) used for the test were Norfloxacin (NOR10 μ g), Ampicillin (AMP10 μ g), Gentamicin (CN10 μ g), Doxycycline (DO30 μ g), Erythromycin (E15 μ g), Trimethoprim-Sulfamethoxazole (SXT1.25 μ g) and Tetracycline (TE30 μ g).

In the current study CNS isolates were susceptible to Gentamicin (100%), Tetracycline (100%), Trimethoprim-Sulfamethoxazole (95%), Norfloxacin (90%), Erythromycin (87%), Doxycycline (70%) and Ampicillin (40%). *Staphylococcus aureus* isolates were also Susceptible to Gentamicin (100%), Erythromycin (100%), and Trimethoprim-Sulfamethoxazole (100%, Norfloxacin (87%), Tetracycline (80%), Ampicillin (55%) and Doxycycline (43%) with trend of decrement in efficacy. *Escherichia coli* was 100% susceptible to Norfloxacin and Trimethoprim-Sulfamethoxazole but less commonly affected by Erythromycin, Ampicillin, Tetracycline and Gentamicin with the potency rate

of $\leq 50\%$. Among anti-biotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole Gentamicin, Doxycycline, Tetracycline and Erythromycin with the efficacy rate of 97%, 94% and 89%, 84% 82% and 70% respectively. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria (55%).

Table 16. Anti-biogram test result.

Bacterial isolates	Frequency	Anti-bio gram susceptibility test in percent						
		NOR10	AMP10	CN10	DO 30	E15	SXT1.25	TE 30
		μg	μg	μg	μg	μg	μg	μg
CNS	52	90	40	100	70	87	95	100
<i>S.aureus</i>	27	87	55	100	43	100	100	80
<i>Esherichia coli</i>	13	100	38	50	88	30	100	45
<i>Klebsiella pneumoniae</i>	8	100	33	98	100	45	96	70
<i>Staphylococcus intemidius</i>	13	100	100	100	89	78	94	100
<i>Enterobacter aerogens</i>	6	100	48	60	100	55	90	78
<i>Bacillus spp.</i>	11	100	48	100	100	70	85	80
<i>Streptococcus spp.</i>	8	100	78	100	80	90	95	100
Total	138	97	55	89	84	70	94	82

NOR= norfloxacin, AMP= ampicillin, CN= gentamicin, DO= doxycycline, E= erythromycin SXT= trimethoprim-sulfamethoxazole and TE=tetracycline

5. DISCUSSION

5.1. Prevalence of mastitis at cow and quarter level

The overall prevalence mastitis at cow level was found to be 73% this is in line with the report of Regasa *et al.* (2010 b) Bishi (1998), who found the prevalence rate of 71% and 69.8%, in dairy farms of Holeta town and Addis Ababa and its vicinity, Ethiopia respectively. This is slightly lower than the report of Matios *et al.* (2009) who reported the prevalence rate of and 64.5% in dairy farms of and Asella, Ethiopia. In contrast, our result was found to be by far greater than the prevalence report of Getahun *et al.* (2008), Gizat *et al.*, (2008), Mekonnen and Tesfaye (2010) and Sori *et al.* (2005), Mungube *et al.* (2005) who reported mastitis prevalence as 33.6%, 56%, 48.1%, 52.78% and 52.3% in the dairy farms of Selalle, Bahir dar, Adama and Sebeta and cross breed dairy cows in Ethiopia respectively.

The overall prevalence of clinical and sub clinical mastitis at quarter level was found to be 8.8% and 28.2% respectively. Matios *et al.* (2009) also reported a sub clinical mastitis of 30.4% in Asella area. Getahun *et al.* (2008) and Mekonnen and Tesfaye (2010), however recorded lower level of sub clinical mastitis prevalence in Selalle (13.6%) and Adama area dairies (22.7%). Regasa *et al.* (2010a), on the other hand reported 34.8% sub clinical quarter wise prevalence which is higher than our finding. Variations in husbandry practices between different areas might, at least, partly explain the difference in prevalence reported by different authors. Quarter level clinical mastitis prevalence in this study was in line with what was reported by Regasa *et al.* (2010b), (10% of clinical prevalence at quarter level). But our finding is higher than those of Mekonnen and Tesfaye (2010) and Getahun *et al.* (2008) who reported quarter wise clinical mastitis prevalence of 2.4% and 0.9% respectively. Matios *et al.* (2009) reported clinical mastitis prevalence level as high as 14.9%.

The 5.2% of the mammary glands examined being blind in our study animals is slightly higher than the report of Matios *et al.* (2009) who found 4.5% of blind quarters. Getahun *et al.* (2008) and Mekonnen and Tesfaye (2010) reported 2.3% and 3.6% blind quarters respectively in their study herds. Today it is a well-accepted fact that agro-ecology, milking practice, breed difference, management practices and other risk factors influence mastitis prevalence, which might explain the observed differences between the reports of different authors in mastitis prevalence. In the present study the higher prevalence level of sub clinical mastitis compared to clinical form; indicate the magnitude of subclinical mastitis problem and low level of attention that given to it in terms of diagnosis and treatment.

5.2. Bacterial isolation and anti-biogram susceptibility test

In this study the highest prevalent bacteria was found to be contagious bacteria of which *coagulase negative staphylococci* (CNS) was found to be dominant followed by *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus intermedius*, *Bacillus species*, *Streptococcus species*, *Klebsiellae pneumoniae*, and *Enterobacter aerogens* with prevalence rates of 37.7%, 19.6%, 9.4%, 9.4%, 8%, 5.8%, 5.8% and 4.3% respectively. Among isolated bacteria, the majority of them were retrieved from sub clinically infected quarters. This finding is comparable with the report of Mekonnen and Tesfaye (2010), Gizat *et al.* (2008) who found CNS as the predominant bacteria among isolates in Adama and Bahirdar dairies, respectively. On the other hand, in different researches, *Staphylococcus aureus* was the most frequently isolated bacteria as per the reports of Regassa *et al.* (2010b), Matios *et al.* (2009), Getahun *et al.* (2008) in dairy farms of Holeta, Asella and Selalle towns, respectively.

The preponderance of contagious mastitis in this study may be ascribed to the lack of proper milking procedure before milking, during the time of milking and post milking. For instance absence of pre and post teat dipping using antiseptics, washing of milker's

hands and using teats secretion as a lubricant of teats at the time of milking which is often practiced in the study area might contributed to the spread of these pathogens from infected teats to healthy ones.

In the present study interestingly environmental bacteria like *Escherichia coli* was isolated in high proportion (9.4%). This is in congruent with the reports of Mekonnen and Tesfaye (2010), Matios *et al.* (2009) who found 7.5% of the total isolates. In contrast, this figure is higher than isolates reported by Regassa *et al.* (2010b), Sori *et al.* (2005) and Getahun *et al.* (2008) who reported 4.57%, 0.75% and 0.5% in different parts of Ethiopia, respectively. The presence of environmental bacteria might be an implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area.

Anti-biogram test for 138 bacterial isolates was performed through a panel of seven antimicrobial drugs. (Oxoid, UK) anti-biotic discs used for the test were Norfloxacin (NOR10µg), Ampicillin (AMP10µg), Gentamicin (CN10µg), Doxycycline (DO30µg), Erythromycin (E15µg), Trimethoprim-Sulfamethoxazole (SXT1.25 µg) and Tetracycline (TE30µg).

In this study CNS isolates were susceptible to Gentamicin, Tetracycline, Trimethoprim-Sulfamethoxazole, Norfloxacin, Erythromycin, Doxycycline and Ampicillin with efficacy rate of 100%, 100%, 95%, 90%, 87%, 70% and 40% in their decreasing order respectively. *Staphylococcus aureus* isolates were also susceptible to Gentamicin (100%), Erythromycin (100%) Trimethoprim-Sulfamethoxazole (100%) Norfloxacin(87%), Tetracycline(80%), Ampicillin(55%) and Doxycycline(43%) with trend of decrement in potency. *Escherichia coli* was 100% susceptible to Norfloxacin and Trimethoprim-Sulfamethoxazole but less commonly affected by Erythromycin, Ampicillin, Tetracycline and Gentamicin with the potency of $\leq 50\%$. Among anti-biotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole Gentamicin, Doxycycline, Tetracycline and Erythromycin with the efficacy rate of 97%,

94% and 89%, 84% 82% and 70% respectively. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria (55%). Anti- bio gram test result in this study is in line with the report of Getahun *et al.* (2008) who found 100% susceptibility to ampicillin and tetracycline in case of *S.intermedius* and as of *S.aures* there was a susceptibility rate of 45.3% for ampicillin. In similar situation with the report by Nibret *et al.* (2011) who indicated tetracycline showed 40% for *Escherichia coli* and 44%for CNS. Whereas in case of CNS it is higher than the report given by Nibret *et al* (2011) who found susceptibility rate of 60% for Erythromycin and 18.5% for *S.aureus* for ampicillin. The difference in susceptibility patters of bacteria to different anti-biotic might be attributed to difference in utilization of anti-microbial agents for treatment regimen and development of resistance due to repeated use of similar anti-biotics in different farms for longer period.

5.3. Associated risk factors and the status of mastitis

Among assessed potential risk factors to the prevalence of mastitis, higher infection rates were observed in cows with advanced age groups, pendulous udder conformation, and multiple parity, poor body condition score, bad hygiene score, high milk producers, early lactation stage, previous exposure to mastitis and blind teats.

The prevalence rate of mastitis at cow level was higher as the age advances; 88%, 77 % and 63% in older, mid age and young cows respectively. There was statistically significant difference among different age groups. This finding is in broad agreement with reports made by different authors in different parts of the country. Demelash *et al.* (2005), Regassa *et al.* (2010b), Mungube *et al.* (2004) who reported age considered as potential risk factor to mastitis and older cows were more affected by mastitis than younger cows,. The increase in prevalence rate with the advancing age may be due to gradual suppression of immune system of the body, structural changes in udder and teats and repeated exposure to milking practices.

Parity was considered as associated risk factor to mastitis in this study in which cows with multiple parities showed higher prevalence (80%) than cows in their first or second lactations (68%). This is in agreement with Mungube *et al.* (2004), Demelash *et al.* (2005) Matios *et al.* (2009), Gizat *et al.* (2008), Girma (2010) and Lakew *et al.* (2009) Molalegn *et al.* (2010) who identified parity as risk factor to mastitis in the study conducted at different parts of Ethiopia.

More cows which had experienced mastitis problem before, were found to be positive to clinical or/and sub clinical form of mastitis at current investigation than non-exposed ones, 89% and 58% respectively. This is comparable with the findings of Demelash *et al.* (2004), Mekonnen and Tesfaye (2010) who indicated cows with previous exposure to udder infection were more likely to be re-infected than those never exposed. This might be attributed to possibility of previously exposed cows remained at carrier state and impotency of drugs used for mastitis treatment in the study area.

Lactation stage was found to be a risk factor to mastitis and the prevalence was highest in early lactation(100%) than mid (43.3%) and late (68%) in this study. which is in agreement with Demelash *et al.* (2004) who reported mastitis prevalence was higher in early lactation (45.8%) than mid lactation (25.8%). In contrary different reports reflected prevalence of mastitis was higher in late stage of lactation than early (Getahun *et al.*, 2008; Gizat *et al.*, 2008). This difference between reports of different authors concerning the stage of lactation in which mastitis is most prevalent, could be attributed to different management practiced in different study areas. The highest prevalence rate during the early lactation is an indication of infection, probably prior to freshening. It may also be reflection of important changes that occur prior to parturition period in endocrine, nutritional and metabolic status which compromise the immunity of the cow. In this stage of lactation, milk yield is increasing this can cause impairment of the immune system due to metabolic stress. When cows are in negative energy balance, body fat is converted to ketone bodies, and hyper ketonemia has been suggested to be one of the most important

factors causing impairment of the udder defense mechanisms and it is likely that the impaired immune system in cows in early lactation results in reduced ability to battle infection (Suriyasathaporn *et al.*, 2000).

Hygiene of the cow in this study was found to be one of the risk factors. Cows with bad hygiene score had higher prevalence rate ((100%) than good hygiene score (54 %) which is in line with Matios *et al.* (2009), Molalegn *et al.*, (2010) and Lakew *et al* (2009) . In case of this investigation there was highly significant difference in cows with pendulous udder conformation than the cows with high up udder conformation with prevalence rate of 89% and 49% respectively. This is in agreement with Sori *et al.* (2005) and Girma (2010) reports. This might be attributed to more exposure to the injurious materials and presence of more contact with contaminated environment.

Interestingly in this investigation, there was strong association between feet problems and presence of mastitis with prevalence rate of 93% and 60% in the cows that had problem of feet than none respectively. This might be partly due to longer time the lame cow spends in horizontal (laying) position that might increase the contact with environmental pathogens and will be prone to mastitis than none affected ones.

Body condition score was considered as risk factor to mastitis in this report. Cows with poor body condition had more prevalence rate (69.2%) than those with good body condition (72%) though the difference was not statistically significant. this is in congruent with the investigation by Mekonnen and Tesfaye (2010) and Mungube *et al.* (2004) who found body condition as one of associated risk factors to mastitis. Animal with poor body condition might experience their immune system not functioning well, thus making them more susceptible to mastitis.

6. CONCLUSION AND RECOMMENDATIONS

This study aimed at determination of the overall prevalence of mastitis and identification of major pathogens with their anti-bio gram sensitivity test. Animal and/or management factors associated with mastitis problems were also undertaken in exotic breed of A.A.T.V.E.T.C. state dairy farm.

The prevalence rate of sub clinical mastitis was found to be higher than clinical form at cow level 56.8% versus 16.2%, and at quarter level 28.2% versus 8.8% and respectively. High prevalence rate of mastitis in this study implied that it is the most serious health problem of the dairy cows of specific farm.

Contagious and environmental mastitis pathogens were retrieved from both clinical and subclinical quarter's milk. Among contagious pathogens the highest prevalent bacteria was found to be *Coagulase negative staphylococci* (CNS) (37.7%) followed by *Staphylococcus aureus* (19.6 %). Whereas *Escherichia coli* (9.4%) was the predominant environmental bacteria followed by *Klebsiella pneumoniae* (5.8%), and *Enterobacter aerogens* (4.3%). The presence of considerable proportion of Enterobacteriaceae suggested that contamination of mammary gland and its environment with animal dung. Moreover, dominant number of contagious microbial agents indicated that improper milking procedures experienced in the farm.

Anti-bio gram profile of the isolated pathogens was performed through a panel of seven antimicrobial agents. Anti-biotic discs used for the test were Norfloxacin (NOR10µg), Ampicillin (AMP10µg), Gentamicin (CN10µg), Doxycycline (DO30µg), Erythromycin (E15µg), Trimethoprim-Sulfamethoxazole (SXT1.25 µg) and Tetracycline (TE30µg). Among anti-biotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole, Gentamicin, Doxycycline, Tetracycline and

Erythromycin with the efficacy rate of 97%, 94% and 89%, 84% 82% and 70% respectively. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria (55%).

Among assessed potential risk factors to the prevalence of mastitis; higher infection rates were observed in cows with advanced age groups, pendulous udder conformation, and multiple parity, poor body condition score, bad hygiene score, high milk producers, early lactation stage, previous exposure to mastitis and blind teats.

Based on the above findings the following points are recommended

- Milkers should be trained on proper hygienic milking methods,
- Regular investigation of mastitis especially sub clinical form should be practiced,
- Mastitis treatments should be preceded with identification of the causative agent and susceptibility test profile of pathogens and
- Culling of old aged and repeatedly infected cows should be done on regular planned basis.
- Management, housing and environmental sanitation should be improved

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8. ANNEXES

ANNEX 1 CMT interpretation

CMT score	Interpretation	Visible reaction	Total cell count
0	Negative	Milk fluid is normal	0-200,000 (0-25% neutrophils)
T	Trace		
		Slight precipitation	(1.5-5) x 10 ⁵ (30-40% neutrophils)
1	Weak positive		
		Distinct	(4-15) x 10 ⁵ (40-60% neutrophils)
2	Distinct positive	precipitation but not gel formation	
3	Strong positive		(8-50) x 10 ⁵ (60-70% neutrophils)
		Mixture thickens with gel formation	
			>5,000,000 (70-80% neutrophils)
		Strong gel that is cohesive with a convex surface	

Source: Quinn *et al.* (1999).

ANNEX 2 Methods used to identify different bacteria (Quinn *et al.* 1994).

A. Staphylococcus species

1. Coagulase test

Tube coagulase test was used for identification of coagulase positive and coagulase negative staphylococcus species.

Procedure

0.5 ml of rabbit plasma was poured in to 10 mm test tube and equal amount of overnight TSB grown presumptive Staphylococcus bacteria was added in the tube and mixed, then incubated at 37 degree Celsius and the result was interpreted after 2-4 hours depending on the formation of clot for positive reaction. *Staph.aureus* and *Staph.intermedius* showed positive result otherwise considered as CNS.

2. Mannitol Salt Agar

Procedure

Mannitol Salt Agar media was prepared, then all gram positive bacteria were inoculated in to the medium and the result was recorded after 24 hrs.incubation period at 37 degree Celsius. Staphylococci species produced yellow colony and yellow medium that indicates mannitol fermentation other bacterial species could not ferment mannitol.

3. Purple base agar with 1% maltose sugar

It was used to differentiate maltose fermenter and non-fermenter staph. species. *S.aureus* produced yellow colony and yellow medium whereas *S. intermedius* ferment maltose slightly and only yellow colony but no color change on the medium.

4. Catalase test

3% hydrogen peroxide was placed on the clean slide with a loop full of bacterial colony and positive reaction is observed by bubble formation.

B. Enterobacteriaceae

<i>Entero bacteriaceae</i>	Methods of identification						Colony characteristics on (Macconkey Agar)
	Gram staining	Citrate	Motility	Indole	Oxidase	sugar fermen- tation (TSI)	
<i>Escherichia coli</i>	G-ve rod	–	motile	+	–	+	Red, not mucoid
<i>Klebsiella pneumoniae</i>	G-ve rod	+	Non motile	–	–	+	Large, red mucoid
<i>Enterobacter aerogenes</i>	G-ve rod	+	motile	–	–	+	pink

Media used are SIM, Simmon's citrate, Triple sugar iron, Macconkey Agar and Kovacs reagent

ANNEX 3 Cow's hygiene score in 1-4 scale

Score	Interpretation	Judgment
1	there is little or no manure above the coronary band and teats	clean
2	minor splashing of manure near the tits and above the coronary band	clean
3	distinct plaques of manure above the coronary band, but with leg hair visible distinct plaques of manure on the lower half of the udder	dirt
4	plaques of manure encrusted on and around the teats, udder, upper leg and flank region	dirt

Source: (Cook, 2002).

ANNEX 4 Body condition score 1-5 scale

Score	Interpretation	Judgment
1	Deep cavity around tail head. No fatty tissue felt between pins. Pelvic bone easily felt. Skin is supple. Ends of short ribs sharp to touch. Upper surfaces can be felt easily. Deep depression in loin.	Poor
2	Shallow cavity lined with fatty tissue at tail head. Some fatty tissue felt under pin bone. Pelvis easily felt. Ends of short ribs feel rounded. Upper surface felt with slight pressure. Depression visible in loin.	Poor
3	No visible cavity around tail head. Fatty tissue is easily felt over whole rump. Skin appears smooth. Pelvis is felt with slight pressure.	Good
4	Folds of fatty tissue are visible around tail head. Patches of fat are present around the pin bones. Pelvis is felt only with firm pressure. Short ribs cannot be felt even with firm pressure. No depression is visible in loin between backbone and hip bone.	Good
5	Tail head is buried in fatty tissue. Skin is distended. No part of pelvis can be felt even with firm pressure. Folds of fatty tissue over short ribs. Bone structures cannot be felt. These cows are good candidates for fat cow syndrome	Good

Sources: (Parker *et al.* 1989).

9. DECLARATION

I, the under signed, declare that, this thesis is my original work, has not been presented for a degree in any university and all the sources of material used for this thesis have been duly acknowledged.

Name: Melesse Etifu Meranga

Signature: _____

Date of Submission: 15 06 2012

This has been submitted for examination with our approval as University advisors

Dr. Mekonnen H/mariam _____

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