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ABBREVIATIONS

ANRS	Amhara National Regional State
CMT	California Mastitis test
CNS	Coagulase negative Staphylococci
FAO	Food and Agricultural Organization
ILDP	Integrated Livestock Development Project
IMI	Intra mammary infection
MIC	Minimal inhibitory concentration
MOA	Ministry of Agriculture
NCCLS	National Committee for Clinical Laboratory Standard
NMC	National mastitis council
O I E.	Organization International Epizootics
SCC	Somatic cell count
TLU	Tropical livestock units
TSST	Toxic shock syndrome toxin
WHO	World Health Organization

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ABSTRACT

A cross _ sectional study was conducted on 322 local and crossbred lactating hand milked small holder cows in and around Gondar from September 2006 to March 2007 to determine prevalence, the causal agents of infection, associated risk factors and tests susceptibility to drugs using California Mastitis Test, clinical inspection of udder, and bacterial culture. Of the total cows examined, 32.6% (322) had mastitis, 0.93 % (3) was clinical and 31.67% (102) sub clinical mastitis. Out of 1249 quarters examined 164 (13.13%) were found to be infected, 7(4.27%) clinically and 157 (95.73%) sub clinically from 1288 quarters examined 39 (3.02%) were blind.

Clinical prevalence at cow level was 0.93% in crossbreds and none in local zebu breeds. Sub clinical mastitis at cow level based on CMT was 28.57% in crossbreds compared to indigenous zebu 3.10% ($P<0.05$). Quarter sub clinical prevalence based on CMT was 16.05% and 5.12% for crossbreds and local zebu, respectively.

Among potential risk factors considered breed, age, parity and stage of lactation were found to affect the occurrence of mastitis significantly ($p< 0.05$). The prevalence of mastitis was significantly higher in Holstein _Friesian crossbreds than indigenous zebu, in the early lactation stage than in the mid lactation stage.

Of 176 CMT and clinically positive udder quarter samples analyzed microbiologically 164 were culturally positive for known mastitis pathogens and 1085 were negative. Of the 164 positive samples isolation rate of *Staphylococcus aureus* 16.5%, Coagulase negative *Staphylococci* 31.1%, *Streptococcus agalactiae* 15.9%, *Streptococcus dysgalactiae* 14.0%, *Streptococcus uberis* 6.7%, Micrococcus spp 7.3%, *Corynebacterium bovis* 2.4%, *Actinomyces pyogens* 1.2%, *Bacillus cereus* 0.6% and *Escherichia coli* 4.3%.

Staphylococcus aureus, Coagulase negative Staphylococcus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Micrococcus species*, Corynebacterium bovis, Actinomyces pyogenes, Bacillus cereus* and *Escherichia coli* were isolated from cases of sub clinical mastitis. Generally, it was found that Sulfisoxazole was the most effective antibiotic

where 91.07% of the total isolates were found susceptible, followed by clindamycin and kanamycin (89.3%), (88.4%) The least effective antibiotics were streptomycin (45.5%) and ampicillin (49.1%). Tetracycline, erythromycin, chloramphenicol, oxacillin, 65.2%, 59.8%, 64.3%, 58.04% respectively, were categorized as drugs of weak efficacy.

Inadequate sanitation of dairy environment, poor animal health services and lack of proper attention to health of the mammary glands were important factors contributing to high prevalence of mastitis. Some recommendations were forwarded to reduce the tend of mastitis in the study area.

Keywords: clinical mastitis / sub clinical mastitis / prevalence / bacterial isolate / antibiotic susceptibility / crossbred cattle / zebu cattle

1. INTRODUCTION AND OBJECTIVES

Ethiopia, a country with a human population of 70 million (annual population growth rate, 2.9%) and a land size of 1.2 million km^2, is very much dependent on agriculture. Livestock represent a major national resource and form an integral part of the agricultural sector. The country has the largest livestock population of any African country with an estimated 35 million Tropical Livestock Units (TLU); this includes 31 million cattle, 42 million sheep and goats, 7 million equines, 1.2 million camels, and more than 53 million chickens and immense bee and fishery resources (Mohammed *et al.,* 2004).

Cows represent the largest proportion of cattle population of the country. According to the Food and Agriculture Organization FAO 42% of the total cattle heads for the private holdings are milking cows. Small- scale dairying is an important agricultural activity in many parts of the developing world, producing a valuable food product and providing a regular income and work for poor households (FAO, 2003). Milk produced from these animals provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country's requirements due to a multitude of factors. A disease of the mammary gland known as mastitis is among the various factors contributing to reduced milk production. Mastitis is one of the most complex diseases of dairy cows that generally involve interplay between management practices and infectious agents, having different causes, degrees of intensity, and variations in duration and residual effects. In Ethiopia, the disease is insufficiently investigated, and information relating to its magnitude, distribution, and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help reduce its prevalence and effects (Tegegne and Yimegnuhal, 2004). This paper describes the results of an investigation made to elucidate the prevalence and risk factors of mastitis in lactating dairy cows in and around Gondar.

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 unacceptable age. Most estimates have shown a 30% reduction in productivity per affected quarter and a 15% reduction per cow/lactation, making the disease one of the most costly and serious problems affecting the

dairy industry worldwide. The disease generally involves on interplay between management practices and infectious agents. Among various infectious agents, bacterial pathogens (the greatest share of these organisms) have been known to be widely distributed in the environment of dairy cows, constituting a threat to the mammary gland (Radostits, 2000).

In Ethiopia, even though the disease of mastitis has been known locally, it has not been studied systematically, resulting in lack of information on the prevalence of disease and associated economic loss (Tegegne and Yimegnuhal, 2004).

The dairy development scheme consists of upgrading the genetic potential of indigenous cattle through cross breeding with exotic breeds, and improving the management. Crossbreed cattle are distributed to farmers. In addition, encouraging the private dairy sector may help increase milk production through intensification. However, mastitis that is a disease of intensification has a high prevalence principally on crossbreed cows as well as in local breeds (Tegegne and Yimegnuhal, 2004).

Many researches have indicated that mastitis remains the most economically damaging disease for the dairy industry worldwide (Owens *et al.,* 1997). According to the study carried out in England and Wales from 1979 to 1982, the average cost of a case of mastitis due to antibiotics used, milk discarded, reduction in quality and quantity of milk produced by a cow was estimated at 60 pound (Blowey, 1990). In United States, economic losses from mastitis have been calculated at approximately 200 USD per cow per year or 2 billion per year for the nation (Seykora and McDaniel, 1985). Mungube, (2001) estimated the economic losses from mastitis in the urban and peri urban areas of Addis Ababa, Ethiopia, to be 210.8 Birr per cow per lactation. Based on these works, most estimates show that on the average the affected quarter suffers a 30% reduction in productivity and the affected cow is estimated to loss 15% its production (Blood and Radostits, 1989).

Milk from mastitic animals is also of a great public health importance by serving as a vehicle in the spread of such diseases as tuberculosis, streptococcal sore-throat and scarlet fever, staphylococcal food poisoning and brucellosis (Schalm *et al.,*1971). Public health hazards

associated with the consumption of antibiotic contaminated milk and milk products cause allergic responses, changes in intestinal flora and development of antibiotic-resistant pathogenic bacteria (Marthe and Ellckson, 1959).

According to the reports of FAO (2003) the total annual national milk production in Ethiopia ranges from 797,900 to 1,197,500 metric ton raw milk equivalents (FAO, 2003). Out of the total national milk production between 85 and 89% is obtained from cattle. However, this amount is by far below the national demand for milk and milk products. A number of reasons could be described for the low annual national milk yield among which mastitis is one of the most important factors. A number of reports indicated that mastitis is a serious problem in the dairy industry in Ethiopia. Nesru (1999) reported a mastitis prevalence rate of 85.6% and 81.2% using CMT and SCC, respectively. According to the same report, out of the CMT positive animals 37.2% did harbor a causal agent for mastitis.

Biru (1989) reported a combined mastitis prevalence of 67.4% at cow level while Bishi (1998) reported a sub clinical mastitis prevalence of 35.5% and 34.3% for small and large scale farms, respectively. Bishi (1998) also reported for clinical mastitis 4.4% and 5.7% prevalence rates at cow level for small and large-scale farms, respectively. The productivity and financial losses due to mastitis in Addis Ababa milk-shed was estimated. The loss due to sub clinical mastitis was 1.51 kg of milk per cow per milking and 3.01 birr per cow per day and for clinical mastitis, the loss was 4.4 kg milk per day and 176 birr in 20 days (Mungube, 2001).

The diagnosis of mastitis in cows is essential in that it can be used to make decisions on the cow regarding appropriate measures to be taken (cull or treat) (Radostits *et al.,*1994a). There are different diagnostic methods for mastitis including indirect somatic cell counts using CMT, indirect chemical test, and clinical examination (Quinn *et al*.,1999). Incubation of standard volume of aseptically collected milk that has been streaked on agar culture medium is used to determine the udder health status as the definite standard diagnostic test for detecting if an infection exists as well as the causative agents (Eriskine, 2001).

The preventive measures against mastitis can be taken in different stages. These are controlling the source of infection controlling the environment and controlling vectors that transmit infection from source to teat end (Blowey, 1990). Due to diverse bacterial etiologies of the disease, a variety of control methods involving hygiene prior to, during and after milking is used to minimize exposure of cows to mastitis causing organisms. Despite these procedures, new cases of mastitis invariably occur and antimicrobial therapy plays a role in the control of bovine mastitis. Treatment decisions are usually made based on previous susceptibility information for the herd in question. (Practically, there are no opportunities of microbial identification and susceptibility reports to be guided as initial therapy decisions). The susceptibility information and susceptibility trend for bacterial species within a herd is important (Owen *et al*.,1997).

In Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects. This paper describes the results of an investigation made to elucidate the prevalence and risk factors of mastitis in lactating dairy cows in Gondar.

In the study area smallholder dairy production is practiced in a mixed crop livestock farming system. Bovine mastitis is suspected to causes great economic losses. There are no previous studies on mastitis in the area.

The objectives of this study are therefore:

- To estimate the prevalence of bovine mastitis in smallholder dairy farms on CMT, bacterial culture and clinical examination in Gondar, Amhara regional State, Ethiopia.
- To determine the major risk factors associated with the prevalence of bovine mastitis.
- To identify the specific bacterial pathogens and determine antimicrobial susceptibility of frequently isolated pathogens.

2. LITERATURE REVIEW

2.1. Definition of Mastitis

According to Nelson and Stephen (1991) the term “mastitis “is an inflammatory reaction of the mammary gland. Infections may be clinical: sub acute, acute, per acute, sub clinical, and chronic depending on the degree of inflammation.

Clinical mastitis is characterized by visible abnormalities in the udder or milk.

Sub acute mastitis is characterized by minor symptoms and alterations observed in the milk such as clots, flakes, or discolored secretions. The quarter may also be slightly swollen and tender.

Acute mastitis is characterized by sudden onset, redness, swelling, hardness, pain and grossly abnormal milk.

Peracute mastitis is fairly uncommon and includes the above symptoms and may also include depression, raised pulse and respiration, loss of muscle coordination, cold extremities, dehydration and diarrhea.

Sub clinical mastitis is far subtler and cannot be detected by visual observation, though conducting laboratory tests can identify it.

Chronic mastitis may begin as any of the clinical forms or as sub clinical mastitis may be intermittent signs of clinical mastitis, usually a progressive development of scar tissue and a change in the size and shape of the affected gland.

2.2. Mastitis causing pathogens

Jabb *et al.* (1993) has indicated that modern techniques of microbial classification have identified more than hundred species, subspecies, and serovars isolated from the mammary gland. *Streptococcus agalactiae* and some types of S*taphylococcus aureus* are obligate parasites of the gland and inevitable pathogens, but the great majority of infections are opportunistic. In addition, some viral diseases like pseudo cowpox, herpes mamilitis, cowpox, papilloma, foot and mouth disease and vesicular stomatitis affecting the epithelium of the teat orifice are mentioned to result in or predispose to mastitis (Hillerton *et al.,* 2001). From the etiological point of view, the pathogenic microorganisms have been classified into two groups, namely, contagious and environmental pathogens based on their distinct characteristics of distribution and interaction with teat and duct (Calvinho *et al.,* 1998). Within the two groups, there are two other sub-divisions as major and minor pathogens. Major pathogens are responsible for more severe cases of mastitis while the minor pathogens are rarely associated with marked leukocytosis and clinical manifestations (Rainard and Peutrel, 1988).

2.2.1. Major pathogens

The major pathogens causing contagious mastitis are *Staphylococcus aureus, Streptococcus agalactiae*, and *Mycoplasma bovis*. These pathogenic microorganisms live and multiply on and in the cow's mammary gland and are spread from cow to cow primarily through milkier' hands and udder wash clothes during milking. Major pathogens causing environmental mastitis are Coliforms *E.coli, Klebseila* species, *Enterobacter aerogens, Streptococcus uberis* and *Streptococcus dysgalactiae* (Watts and Salmon, 1997).

The predominant infectious agents of the mammary gland in most countries are *Staphylococcus aureus* followed by *Streptococcus agalactiae* (Radostits *et al.,* 1994b). In a study carried out in Zimbabwe by Perry *et al.* (1987) *Staphylococcus aureus* was the most frequently isolated bacteria from both clinical and sub clinical mastitis. (Sargeant *et al.,* 1998) reported isolation rates of 6.8%, 0.7%. 14.1% 17.2%, 1.7% and 28.7% for *Staphylococcus aureus, Streptococcus agalactiae*, and other *Streptococci* species, respectively, from 834 mastitic milk samples collected from clinical cases.

2.2.2. Minor pathogens

In this group Coagulase negative staphylococcal species, *Actinomtyces bovis, Bacillus cereus,* and *Serratia marcescens* are included (Brooks *et al.,* 1983) Coagulase negative staphylococci (CNS) consisting of a variety of *Staphylococcus* species and *Corynebacterium bovis* are contagious minor pathogens. The agents simply colonize the teat streak canal but do not cause a clinical disease (Harmon and Lang Loris, 1988). *Nocardia asteriodes, Serratia marcescens, Prototheca zopfii, Leptospira interrogan (serovar Pomona)* and *pseudomonas aerugenosa* are environmental pathogens of bovine mastitis (Quinn *et al.,* 1999).

2.3. Diagnosis

Clinical mastitis is recognized by appearance of abnormal milk, gland swelling and or illness. Sub clinical mastitis is characterized by normal milk and hence requires indirect tests to detect for example Somatic Cell Count (SCC), California Mastitis Test (CMT) and culture (Dorgent-Mellna *et al.,* 1988)

2.3.1. Somatic cell count (SCC)

The somatic cell count of milk is used to identify cows that are infected and to estimate the prevalence of mastitis in an entire herd. Milk from normal or uninfected cows will usually have a somatic cell count in the range of 50,000 to 200,000 (Larsen, 2000). The two most commonly used methods are the coulter milk cell counter, which counts particles as they flow through an electric field, and the fossomatic milk cell counter, which stains cells with a fluorescent dye and then counts the number of fluorescing particles (Schalm *et al.,* 1971). Somatic cell count is internationally recognized as a parameter for assessing milk quality and udder health. Today, most markets in developed countries pay a premium for low SCC (Hogan *et al*., 1988)

Dohoo and Meek (1982) reported 250,000 and 300, 000 cells /ml threshold to quarter and cow, respectively. Emanuelson (1997) used threshold of 200,000 cell/ml for cow level to monitor herd mastitis in Sweden. The thresholds for milk quality have no relation to the definition of udder health categorization. At present a threshold of 100,000 cells /ml can be assumed an internationally accepted definition of udder health (Hamann, 2003).

Less than 200,000 cells/ml for a cow and less than 130,000 cells/ml for bulk tank milk were reported by Larsen (2000). In Ethiopia, there is no sufficient information relating to this issue.

2.3.2 California mastitis test (CMT)

The California Mastitis Test estimates the somatic cell content of milk. The scores are related broadly to the number of somatic cells in milk. Somatic cell numbers in milk tend to increase during milking, and remain high for several hours afterwards, even in uninfected quarters. For reliable results, tests should be conducted just before milking after stimulating the cow and discarding the foremilk (Auldist *et al.*, 1995). The CMT reagent reacts with genetic material of somatic cells present in milk to form jel. A squirt of milk, about 2 ml from each quarter will be placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent will be added to each cup. A gentle circular motion will be interpreted according to Schalm *et al*. (1971) and Quinn *et al.* (1994) (Annex 2). The test results are interpreted subjectively as a negative, trace, $1^{+,}$ 2^{+}or 3^{+} inflammatory responses based on the viscosity of the gel formed by mixing the reagent with milk (Radostits *et al.,*1994b). The CMT reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time.

2.3.3. Culture

In working with a mastitis problem herd, it is desirable to know the types of microorganisms causing infections. This is performed by microbiologic culture of milk samples from individual quarters or of composite samples from all four quarters of individual cows. The culture results are important for an adequate understanding of specific herd problems, for making recommendations for therapy, and for making culling decisions on individual cows (Radostits *et al.,* 2000). Most of the bacterial pathogens causing mastitis grow on ox or sheep blood agar. Gram- negative isolates were inoculated onto Mac Conkey's and then evaluated by standard procedures. Edwards' medium is highly selective for *Streptococci* and also acts as an indicator medium for haemolysis and for hydrolysis of aesculin (Dinsmore *et al.,* 1992). Interpretation was made as provided by National Mastitis Council (1990). The culture is considered negative if no growth occurs after 72 hours of incubation. The isolation of only a few colonies may be indicative of a true intramammary infection but may also be the result of contamination. Frequent isolation of several

types of bacteria from individual quarter samples suggests poor collection technique. If three or more colony types are isolated from a quarter sample, contamination should be assumed and the results disregarded unless *Staphylococcus aureus* and *Streptococcus agalactiae* is present. When contamination of a sample is suspected, a new sample should be obtained if at all possible (Carter, 1984).

2.4. Control of bovine mastitis

Different mastitis organisms require different treatment regimens and control strategies like mastitis therapy, controlling contagious mastitis and controlling environmental mastitis. Timely and accurate milk bacteriological culture results would benefit producers and veterinarians. Udder health management during the dry period is an integral period of elimination of existing and prevention of new IMI (Winkler, 1982).

2.4.1. Mastitis therapy

Successful treatment of clinical mastitis requires a history of the herd, identification and susceptibility pattern of the bacteria involved as well as relevant information on the milking management. Intra mammary drug formulation is a suitable route for administrations of long acting antimicrobial preparation at “dry off “as part of mastitis control programmed (Quinn *et al.,* 1999).

Eriskine (2001) has suggested that the prevalence of *Streptococcus agalactiae* intra mammary infection can be reduced rapidly by “blitz” treatment, with this method an entire herd, or more economically, all the culture positive cows in a herd, are treated with antimicrobials. The most efficacious and cost effective regimen is to use intra mammary β –lactam therapy. Cure rates can often be from 75% to 90% and are economically beneficial (Eriskine *et al.,* 1988). Studies of treatment efficacy of *Staphylococcus aureus* mastitis have found cure rates of 25% to 55% of infected quarters in experimental infections that were evaluated for 21 to 60 days after infection (Owens *et al.,* 1998). However, natural infections are usually of longer duration before therapeutic intervention is used and thus more refractile to therapy.

Intra mammary Cefoperzon for the treatment of clinical *Staphylococcus aureus* mastitis led to bacteriologic cures for only 39% of the cases, as measured 14 days after treatment (Wilson *et al.,* 1986).

In Ethiopia, (Hussein *et al*.,1997) reported that out of five isolates of *S. aureus* four were sensitive to penicillin and streptomycin, all to erythromycin, three to chloramphenicol and ampicilline but most isolates (four) were less sensitive to tetracycline. Therefore, therapy of clinical intramammary infection may have the best probability of success by including parenteral in addition to intramammary therapy; preferentially it should be administered for periods long enough to ensure drug levels above minimal inhibitory concentration (MIC) to allow effective killing of the pathogens (Eriskine, 2001).

2.4.2. Controlling contagious mastitis

Proper milking procedures are important for the prevention of mastitis and for ensuring complete milk removal from the udder. Milking may begin with a check of all quarters for mastitis, strip milk onto the floor in a milking parlor or flat barn. Any cows that show clinical mastitis should be examined and appropriate action taken (Pearson, 1979). The procedure for pre dipping involves washing teats with water then dried with an individual towel and dipped or sprayed with an effective germicidal (Owens *et al*., 1997). Post milking teat disinfection; treat all quarters of all cows at drying off with antibiotic products specifically designed for dry cow therapy and culling chronically infected cows (Watts, 1988).

2.4.3. Controlling environmental mastitis

Cow environment should be as clean and dry as possible. Cow should not have access to manure, mud or pools of stagnant water. Calving areas must be clean. Adequate accommodation for cows, good udder preparation, and premilking disinfection are recommended (Sol *et al*., 1997).

2.5. Zoonotic significance of milk and milk Products

Milk from mastitic animals is also of great public health importance by serving as a vehicle in the spread of such diseases as tuberculosis, Streptococcal sore throat and scarlet fever,

Staphylococcal food poisoning and brucellosis (Schalm *et al*., 1971 and Quinn *et al*.,1994). Zoonoses transmitted through milk and dairy products can either originate from the dairy cow directly or from the environment at any stage from production to consumption. The presence of *Staphylococcus aureus* in market milk may present a degree of risk to the consumer because of the organisms' capacity to produce enterotoxins and a toxic shock syndrome toxin (TSST), which causes serious food poisoning (WHO/FAO/OIE Report, 1999). Public health hazards associated with the consumption of antibiotic contaminated milk and milk products cause allergic responses, changes in intestinal flora and development of antibiotic-resistant pathogenic bacteria. Food borne pathogens which cause widespread disease in dairy animals such as *Mycobacterium bovis, Brucella abortus* and *Brucella melitensis*, continue to be as serious animal and public health burden in developing countries and require national long term eradication efforts based on annual testing and aimed at removal of infected animals from the human food chain (Ryser and Marthe, 1989).

2. 6. Epidemiology of bovine mastitis in Ethiopia

2. 6.1. Prevalence

A number of epidemiological studies of bovine mastitis are conducted in Ethiopia. Hussien *et al.* (1997) reported the prevalence of clinical and sub clinical mastitis to be 1.9% and 5.3% on cow basis, respectively, 1.9% and 7.4% on quarter basis respectively in the central regions of Ethiopia. Bishi (1998) reported the over all prevalence to be 30.2% and 5.5% for sub clinical and clinical mastitis, respectively, in a study conducted in urban and peri urban dairy production systems in and around Addis Ababa. In a study conducted at Repi and Debre-Zeit dairy farms, out of 186 lactating cows, 40 (21.5%) were clinically affected and 71 (38.%) Sub clinically infected (Workineh *et al.,* 2002). According to Tesfu *et al.* (1999) a survey carried out on mastitis in dairy herds of the Ethiopian central highlands, the prevalence of clinical mastitis and sub clinical mastitis on cow basis was 1.2% and 38.9%, respectively. In another investigation by Lemma *et al.* (2001) on crossbred dairy cows in Addis Ababa milk shed, clinical mastitis was the second most frequent disease next to reproductive diseases, in which 171 cows out of 556 were found to be affected. In the same study area Mungube (2001) reported an overall prevalence of 46.6% for sub clinical mastitis at cow level and 27.8% at quarter level. This variation could

result from differences in environment and management (Kerro,1997). In another study in the same area, Lemma *et al.* (2001) reported that clinical mastitis was the second most frequent disease next to reproductive diseases. Variation of the prevalence of mastitis due to differences in environment and management were reported by Oudessa (1997).

A study was done to quantify mastitis in Bahir Dar milk shed. Sub clinical mastitis was more important when compared to clinical mastitis. Crossbred cows were affected more than local zebu. The pathogens found involved are Coagulase positive staphylococcus (CNS), *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Micrococcus* species, *Corynebactreium bovis, Actinomyces pyogens, Bacillus cereus,* and *Streptococcus intermedius*. Among these, the most frequent isolates were CNS (49.63%). *Staphylococcus aureus* has a prevalence of 17.78%, followed by *Streptococcus agalactiae* (8.5%) and *Streptococcus dysgalactiae* (6.67%) (Gizat, 2004).

2.6.2. Incidence

There are also some limited reports on the incidence of mastitis. A survey on bovine mastitis in milking cows at Alemaya University Dairy Farm by Tefera (2001) for 6 years (from 1993 to 1998) revealed an overall clinical mastitis incidence of 34 cases /100 cows per year. Nesru (1999) reported incidence rates of 1.33% and 11.2% per cow per month for clinical and sub clinical mastitis, respectively, in urban and peri_urban dairy farms in Addis Ababa.

2.6.3. Economic loss

The overall prevalence of mastitis due to *Staph .aureus* is much higher than *Strep. agalactiae*, the risk of new infections is the major concern mastitis may cause severe economic losses resulting partly from decreased milk production, discarded milk from cows with clinical mastitis and treated cows, replacement cost of culled cows extra labor required for treatment and monitoring, veterinary service for treatment and control (Mungube, 2001). In economic terms, mastitis can generally be considered the most serious disease of dairy cows. If sub clinical and chronic infections are treated with antimicrobials, the milk losses resulting from drug residues are similar to those for clinical infections. Sub clinical infection reduces milk production but the estimates for the fall in production vary greatly 9-45% per infected quarter.

A recent study has revealed that mastitis accounted for 26% of the total cost of all dairy cattle diseases (Nelson and Stephen, 1991).

In Ethiopia, there is limited information on the economic loss due to mastitis. However, the few data available indicate that the loss is significant. Mungube (2001) estimated the economic loss from mastitis in the urban and peri urban areas of Addis Ababa. He used milk production losses, treatment costs, withdrawal and culling losses as parameters for calculating losses. This loss was found to be 210.8 Birr per cow per lactation. In this study, loss due to culling, milk loss, treatment, and withdrawal contributed 49%, 38.4%, 9.3% and 3.3% to the total mastitis losses, respectively. Milk production losses contributed 38.4% of the total losses, sub clinical mastitis contributing 94% and clinical mastitis 6% of the milk losses. Sub clinical mastitis contributed 36.1% of the total losses, which is primarily due to reduced milk production (Kassa *et al.,* 1999). Bishi (1998) also reported the economic losses from clinical and sub clinical mastitis to be approximately 270 Ethiopian birr per cow per lactation

3. MATERIALS AND METHODS

Milk samples collected from cows affected with clinical and sub clinical (positively screened with CMT, were cultured at the microbiology laboratory of the faculty of Veterinary Medicine Gondar University. The milk samples were examined bacteriologically following standard procedures. About 10 ml of milk sample was streaked onto tryptose blood agar base enriched with 7% sheep blood. Blood agar plates were incubated aerobically at 37 ^{0}C for up to 72 h. The plates were examined for growth, colony morphology and haemolytic characteristics at 24, 48 and 72 h after inoculation. Bacteria on culture positive plates were identified according to their Gram-stain reaction, colony morphology, and haemolytic characteristics and catalase test. *Staphylococcus, Micrococci* and *Streptococcal* species were identified following standard methods. Gram- negative isolates were inoculated onto Mac Conkey's and then evaluated by standard procedures.

3.2. Study area

The study was conducted in and around Gondar town, North Gondar Administrative Zone of Amhara National Regional state (ANRS). Gondar is located in the north- western part of Ethiopia, 710 km North West of Addis Ababa. The study area is found at 12^{0} 40^{1}N Longitude and 37^{0} 45^{1}E, Latitude with an altitude range of 1802-2200 meters above sea level. The soil type falls into three categories: Heavy black clay soil, loam brown and red soil and sandy loam soil. The ranges of maximum and minimum temperature vary between 22-30.7^{0}c and 12.3-17.1^{0}c, respectively. The region receives a bimodal rainfall, the average annual precipitation rate being 1000mm. The short rains occur during the months March, April and May while the long rains extend from June through September (MOA, 2004).

3.1.Study population

3.3.1. Study animals and husbandry practices

The study population comprises of approximately 322 crossbred and lactating Zebu cows in and around Gondar town managed under semi-intensive and extensive production system. In the

semi-intensive production system, the animals are mainly composed of crossbred cattle. They are kept indoors and graze in the field occasionally. They are supplemented with concentrates in addition to hay. On the other hand, the extensive production system that consists of local breeds that depend for feed on grazing at the field with minor supplementation in the evening when they come back home. Local dairy cows are managed under traditional and extensive husbandry systems. The animals are relatively smaller in size and have small udder and short teats. The average daily milk production from individual cows was relatively low (4 to 5 liters). Crossbred dairy cows are often managed under a small-scale, semi-intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and crop by products and are maintained usually in separate stalls a short distance from each other in a house. This type of dairy husbandry system is increasingly becoming an important source of milk supplies to households and a means of income generation in urban and peri-urban areas of Gondar. Manure removal is generally made on a daily basis. Although milking is done by hand, pre-milking and post-milking hygienic procedures, such as udder washing and drying are not practiced. Cows are dried off at late-lactation abruptly.

3.3. Study design

3.3.1 Prevalence study

The study was a cross sectional study conducted from October 2006 to March 2007 in and around Gondar town. Grades of the CMT were evaluated and the results graded as 0 and 1 for negative and 2 and 3 for positive. Disease identification was made based on clinical examination, nature and appearances of milk secretion, and reaction to CMT. Accordingly, milk with pus flakes, clots, or blood-tinged watery secretion, yet no visible or palpable changes in mammary quarters, and acute mastitis with signs of systemic involvement were diagnosed as clinical mastitis. Sub clinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively. This study also incorporated prevalence of blind/blocked quarters.

$$\text{Overall prevalence of sub clinical Mastitis} = \frac{\text{Number of cows with sub clinical mastitis} \times 100\%}{\text{Total number of lactating cows}}$$

$$\text{Quarter prevalence} = \frac{\text{Number of positive quarters x 100\%}}{\text{Total number of quarter screened}}$$

$$\text{Prevalence of blind/blocked quarters} = \frac{\text{Number of quarters blind/blocked x 100 \%}}{\text{Total number of quarters in examined cows}}$$

3.3.1.1 Sample size and sampling method

Sample size was determined at 95% confidence interval, 5% precision and from previous studies in similar study area (Workineh *et al.,* 2002), with an expected prevalence of 30% .The sample size was determined by using the formula for simple random sampling (Thrusfield , 1995).

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

Where:

n = required sample size,

Pexp = expected prevalence,

d = desired absolute precision

The sample size thus determined was 322 animals to be drawn from the study population. However, because of the difficulties of recording all lactating cows, sampling frame, and considering the number of cows in each household on the average to be 2 cows, the number of households selected was determined by dividing sample size with herd size, in this case 322/2 = 161. Hence, these study 161 households were included.

3.3.1.2 Data collection

A questionnaire was developed, pre tested and administered to the owners of the animals. Data on each cow was collected in a format designed for this purpose (Annex 1). Relevant information was collected on cow history, housing system, milking practice, drug usage and other management practices. Risk factors considered were breed, age, parity, stage of lactation, tick load or lesion and husbandry system. Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub clinical mastitis.

3.3.1.3 Sampling equipment

Samples were taken in sterile glass vials and closed with screw caps. The vials were marked with a permanent marker, so that the markings were easy to read when the vials were placed in a rack. The vials were marked before sampling. The surface of the teat ends were sterilized by wiping with clean cotton dipped in 70% alcohol. An insulated cool box was used for transporting samples.

3.3.1.4 Clinical inspection of the udder

The clinical inspection of the udder was done in the following way. The udder was first examined visually and then by palpation to detect fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Mammary quarters often became blind when exposed to repeated infections and little or no treatment was provided. Information relating to the previous health history of the mammary quarters and causes of blindness was obtained from interviews with owners of the farm. Viscosity and appearance of milk secretion from each quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury and ticks. Injuries caused by ticks and vigorous calf suckling were described based on location, size, and nature. Injuries caused by ticks were identified as indurate necrotic lesions following detachment of the ecto parasites; these could be with or without abscess formation. Injuries caused by vigorous calf suckling were identified as circumscribed lesions around the teats.

3.3.1.5 Preparing udders and teats

Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton or gauze sponge moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing was avoided, the teats on the far side of the udder first, then those on the near side. A separate pledged or sponge was used for each teat. Scrubbing was continued until a new surface of the cotton or sponge remains clean.

3.3.1.6 Collection of milk samples

Procedures for collecting milk sample were according to (Schalm *et al*., 1971; Sears *et al*., 1993) and Quinn *et al*., 1994). Strict aseptic procedures were used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment. Teats towards sample collection were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position, 15ml of milk was collected into the vial.

3.3.1.7 Time of sample collection

Samples for culture were collected before milking that was most convenient under the management conditions of the individual herd.

3.3.1.8 Handling and storing samples

After collection, samples for cultures were placed in racks for ease of handling and held in an icebox, properly packed and kept cold**.** They were processed as soon as possible.

3.3.2 California mastitis test (CMT)

The California mastitis test was carried out as screening test for selections of samples for culture following the method described by Schalm *et al.* (1971) and Quinn *et al.* (1994). A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was

applied to the mixtures, in a horizontal plane for 15 seconds. The reaction was interpreted according to Schalm *et al.* (1971) and Quinn *et al.* (1994) (Annex 2).

3.3.3 Bacterial isolation

In the milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature for about an hour and then mixed by shaking. Bacteriological examination of the milk was carried out following standard procedures of Carter *et al.* (1984), Sears *et al.*(1993), Quinn *et al* .(1994). One standard loop (0.01ml) of milk sample was streaked on 7% blood agar. The inoculated plate then was incubated aerobically at 37 ^{0}C for about 24, 48 and up to 72 hours to rule out slow growing microorganisms. For primary identification, colony size, shape, color, hemolytic characteristics, grams reaction and catalase production were used. This was conducted at Gondar University Microbiology Laboratory, Gondar. Suspected colonies were isolated onto blood agar plates for further investigation, for confirmation biochemical tests were used after sub culturing isolated distinct colonies onto a nutrient agar (Annex 3).

3.3.4 Antibiotic susceptibility test

The antimicrobial resistance patterns of the isolates were determined using the Kirby Bauer disk diffusion technique. The disks were impregnated with the following antibiotics: tetracycline, erythromycin, kanamycin, chloramphenicol, streptomycin, ampicillin, sulfisoxazole, oxacillin, and clindamycin.

Discs were stored under refrigeration to ensure maintenance of their potency. Well-isolated colonies of the same morphologic type were inoculated into 5ml of a nutrient broth incubated at 37°c for 5 hours until a visible turbidity appeared. The turbidity was compared to the 0.5 Mc farland standards. Mueller Hinton agar was used as plating medium. 15 minutes after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates with sterile forceps. All discs were gently pressed down onto the agar with forceps to ensure complete contact with the agar surface; the plates were inverted and then aerobically incubated for 18 hours at 37°c. The diameters of the zones of complete inhibition were measured to the nearest whole millimeter using a ruler. Zones of inhibition for individual antimicrobial agents

were translated into susceptible, intermediate, and resistant categories by referring the recommended NCCLS interpretative standards (Annex 4).

Retrospective data were compiled on the type of antibiotics used to treat mastitis and other infectious diseases in the region. Specifically the antibiotics used to treat clinical mastitis cases were gathered from clinical casebook records.

3.4. Data Analysis

All data collected were entered into Microsoft excel spreadsheet. For analysis of the data SPSS version 10 and EPI-INFO 2000 software's were used. In this chi-square test, odds ratio, confidence intervals and logistic regression (linear model) were calculated.

4. RESULTS

4.1. Prevalence

A total of 322 lactating cows (57 indigenous zebu and 265 Holstein x zebu) crosses in small holder dairy farms in Gondar milk shed were investigated from September 2006 to March 2007 cross sectionally to determine the magnitude of mastitis. Out of 1288 quarters examined 39 (3.02%) were blocked. Clinical prevalence at cow level was 3 (0.93%) in cross breeds and none in local zebu breeds (Table 1).

Table 1. Prevalence of clinical mastitis at cow level in crossbred and local zebu lactating cows

Breed	No. of animals examined	Status of clinical mastitis		
		Positive	Negative	Prevalence % & 95% CI
Cross	265	3	262	1.13 (0.0,2.4)
Local zebu	57	0	57	0
Total	322	3	319	0.93 (0.0,1.9)

The prevalence of sub clinical mastitis was determined by CMT and by microbiological culture (Table 2). Sub clinical mastitis at cow level was 34.72% in cross breeds and 17.54% in indigenous zebu using CMT (Table 2). Based on culture sub clinical mastitis prevalence at cow level was 30.57% in cross breeds and 15.78% in indigenous zebu (Table 3).

Table 2: Prevalence of sub clinical mastitis at cow level using CMT in crossbred and local zebu lactating cows

Breed	No. of animals Sampled	Status of sub clinical mastitis		
		Positive	Negative	Prevalence % & 95% CI
Cross	265	92	173	34.72 (28.98, 40.45)
Local zebu	57	10	47	17.54 (7.67, 27.42)
Total	322	102	220	31.68 (26.59, 36.78)

Table 3: Prevalence of sub clinical mastitis at cow level using culture in crossbred and local zebu lactating cows

Breed	No. of animals Sampled	Status of sub clinical mastitis		
		Positive	Negative	Prevalence % & 95% CI
Cross	265	81	184	30.57 (25.02,36.11)
Local zebu	57	9	48	15.78 (6.32,25.26)
Total	322	90	232	27.95 (23.05,32.86)

Based on CMT the prevalence of quarter level sub clinical mastitis in cross breed and local zebu cows was 16.05% and 5.12% respectively. Based on culture the prevalence of quarter level sub clinical mastitis in cross breed and local zebu cows was 14.99% and 4.19%, respectively (Table 4 and Table 5).

Table 4: Prevalence of sub clinical mastitis at quarter level using CMT in crossbred and local zebu lactating cows

Breed	No. of Quarters Sampled	**Status of sub clinical mastitis**		
		Positive	Negative	Prevalence % & 95% CI
Cross	1034	166	868	16.05 (13.82 ,18.29)
Local zebu	215	11	204	5.12 (2.17, 8.06)
Total	1249	176	1073	14.09 (12.16,16.03)

Table 5: Prevalence of sub clinical mastitis at quarter level using culture in crossbred and local zebu lactating cows

Breed	No. of Quarters Sampled	**Status of sub clinical mastitis**		
		Positive	Negative	Prevalence % & 95% CI
Cross	1034	155	879	14.99 (12.81,17.17)
Local zebu	215	9	206	4.19 (1.51, 6.86)
Total	1249	164	1085	13.13 (11.26,15.00)

Both at cow and quarter level, prevalence were high in cross breeds compared to locals. The difference was statistically significant ($p < 0.05$).

4.2. Risk factors affecting prevalence of mastitis

Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Six variables were considered as potential risk factors for the occurrence of sub clinical mastitis in this study. These risk factors were (breed, age, parity, lactation stage, and tick load or lesion and husbandry practice). The association of mastitis with the risk factors was investigated using chi-square (χ^2) and odds ratio (OR).

Table 6: Risk factors for the occurrence of sub clinical mastitis Odds ratio and P-values as estimated by the logistic regression equation.

Variable	Odds ratio	P-value
Breed	2.331	0.000
Age	1.344	0.005
Parity number	1.566	0.009
Stage of lactation	1.710	0.000
Tick /or lesion	_	0.065
Husbandry system	_	0.715

Breed, age, parity and stage of lactation were statistically significant ($p<0.05$). Crosses of zebu with Holstein Frisian cows were affected (71.8%) at higher rate than zebu breeds (28.2%); the difference is statistically significant ($p<0.05$). Older cows greater than 8 years were more affected sub clinical mastitis (79.6%) than younger cows (20.4%); the difference is statistically significant ($p<0.05$). Prevalence of sub clinical mastitis in cows delivered 1 to 3 calves was (14.2%), 4 to 6 calves (31.6%) and greater than 6 calves (54.2%); the difference is statistically significant ($p<0.05$). Mastitis prevalence was highest in early lactation (47.3%) and also higher in late stage of lactation (39.9%) but low in mid lactation (12.8%); the difference is statistically significant ($p<0.05$ (Table 6).

4.3. Bacterial isolation

The major bacteria isolated in this study were S*taphylococcus aureus* (16.5%), *Streptococcus agalactiae* (15.9%), *Streptococcus dysgalactiae* (14.0%), *Streptococcus uberis* (6.7%), *Escherichia coli* (4.3%) and *Actinomyces pyogenes* (1.2%). Under minor pathogens CNS accounted for (31.1%), *Micrococcus* species (7.3%). *Corynebacterium bovis* (2.4%) and *Bacillus cereus* (0.6%). The prevalence rates of various bacterial pathogens in local zebu and crossbred lactating cows are shown in (Table 7).

Table 7: Frequency distribution of bacteria isolated from mastitic milk

Isolate	Frequency	Percentage
Staphylococcus aureus	27	16.5
Coagulase Negative Staphylococci	51	31.1
Streptococcus agalactiae	26	15.9
Streprococcus dysgalactiae	23	14.0
Streptococcus uberis	11	6.7
Micrococcus spp.	12	7.3
Corynebacterium bovis	4	2.4
Actinomyces pyogenes	2	1.2
Bacillus cereus	1	0.6
Escherichia coli	7	4.3
Total	**164**	**100**
Mixed Growth	16	
No Growth	1069	
Total	**1249**	

The majority of the isolates in this study were from cases of sub clinical mastitis. Out of 12 quarters affected clinically, bacteria were isolated from 7 quarters only; the remaining 5 yielded no bacteria. Isolates from clinical cases were *Escherichia coli*, CNS and *Streptococcus agalactiae* (Table 8).

Table 8: Frequency of bacteria isolated from mastitic milk in cross breed and local zebu lactating cows

Isolate	Local breed		Cross breed		Total
	Frequency	%	Frequency	%	
Major Pathogens					
Staphylococcus aureus	3	33.33	24	15.48	27
Streptococcus agalactiae	1	11.11	25	16.13	26
Streptococcusdysgalactiae	1	11.11	22	14.19	23
Streptococcus uberis			11	7.08	11
Actinomyces pyogens			2	1.29	2
Escherichia coli			7	4.52	7
Minor Pathogens					
Coagulase Negative Staphylococci	2	22.22	49	32.26	51
Micrococcus species	2	22.22	10	6.45	12
Corynebacterium bovis			4	2.58	4
Bacillus cereus			1	0.66	1
Total	**9**	**100**	**155**	**100**	**164**

4.4 Antibiotic susceptibility results

The result of bacteriological analysis are presented in table 8 from a total of 164 isolations were 27 *S. aureus*, 51 CNS, 26 *Str. agalactiae*, 23 *Str.dysgalactiae* 11 *Str .uberis*, 12 *Micrococcus* species, 4 *C. bovis*, 7 *E. coli,* 2 *A. pyogenes* and 1 *B. cereus* were isolated. Antimicrobial susceptibility test was done on 112 isolates. The distribution and percentage of isolates tested for antibiotic susceptibility are shown (Table 9).

Staphylococcus aureus was highly sensitive to kanamycin (100%), sulfisoxazole (100%), clindamycin (88.9%), chloramphenicol (81.5%), and tetracycline (70.4%); and was resistant to ampicillin (only 18.5% susceptibility). In this study, sulfisoxazole and chloramphenicol were most effective on *S. aureus* isolates. Coagulase Negative *Staphylococci* was more susceptible to sulfisoxazole (88%), kanamycin (84%) tetracycline (80%) and clindamycin (76%); however CNS was resistant to ampicillin (44%), chloramphenicol (44%), streptomycin (44%), and oxacillin (40%). *Streptococcus agalactiae* was highly susceptible to sulfisoxazole (100%), clindamycin (100%) and susceptibility to streptomycin was 50%. *Streptococcus dysgalactiae* showed susceptibility to clindamycin (100%), ampicillin and kanamycin (80%), and was resistant to streptomycin (100% resistant). *Streptococcus uberis* was most sensitive to chloramphenicol (100%), sulfisoxazole (100%), oxacillin (100%), clindamycin (100%), kanamycin (85.7%) and least responsive against erythromycin (20% susceptibility). *Micrococcus* species was highly sensitive to erythromycin (100%), kanamycin (100%) and clindamycin (100%) but poorly susceptible to streptomycin and chloramphenicol (50%).

Escherichia coli was found highly susceptible to chloramphenicol (100%), kanamycin (100%) and sulfisoxazole (100%), however was highly resistant to erythromycin (only 20% susceptibility) to ampicillin and tetracycline (40%). *Corynebacterium bovis* was found highly susceptible to tetracycline (100%), kanamycin (100%) and sulfisoxazole (100%), but resistant to oxacillin (25%). *Actinomyces pyogenes* was found highly susceptible to sulfisoxazole (100%), but was 100% resistant to tetracycline, chloramphenicol and oxacillin. *Bacillus cereus* was susceptible to ampicillin (100%), tetracycline (100%), kanamycin (100%), sulfisoxazole (100%),

and clindamycin (100%). However, these 100% bacteria were resistant to erythromycin, chloramphenicol, and oxacillin.

In general it was found that for the bacterial isolates tested for antimicrobial susceptibility, sulfisoxazole was the most effective antibiotic where 91.07% of the total isolates were found susceptible, followed by clindamycin and kanamycin with susceptibility of (89.28%) and (88.4%), respectively. The least effective antibiotics were streptomycin (45.5%) , ampicillin (49.1%). Tetracycline, erythromycin, chloramphenicol, and oxacllin have susceptibility of 65.2%, 59.8%, 64.3%, and 58.04%, respectively.

Table 9: Results of antibiotic susceptibility tests on bacteria isolated from milk samples obtained from cows with Mastitis.

Bacterial Isolates	**N**	**TET**	**CMP**	**S**	**Oxa**	**Amp**	**Su**	**ERY**	**K**	**CI**
S. aureus	27	19(70.4)	22(81.5)	14(51.8)	22(81.5)	5(18.5)	27(100)	14(51.8)	27(100)	24(88.
CNS	25	20(80)	11(44)	11(44)	10(40)	11(44)	22(88)	15(60)	21(84)	19(76)
Str. agalactiae	20	12(60)	16(80)	10(50)	15(75)	12(60)	20(100)	16(80)	16(80)	20(100
Str.dysgalactiae	15	6(40)	6(40)	0(0)	4(26.7)	12(80)	10(66.7)	9(60)	12(80)	15(100
Str. uberis	7	4(57.1)	7(100)	4(57.1)	7(100)	4(57.1)	7(100)	3(20)	6(85.7)	7(100)
Micrococcus spp	6	5(83.3)	3(50)	3(50)	4(66.7)	4(66.7)	4(66.7)	6(100)	6(100)	6(100)
E. Coli	5	2(40)	5(100)	4(80)	2(40)	2(40)	5(100)	1(20)	5(100)	4(80)
C. bovis	4	4(100)	2(50)	3(75)	1(25)	3(75)	4(100)	2(20)	4(100)	3(75)
A. pyogenes	2	0(0)	0(0)	1(50)	0(0)	1(50)	2(100)	1(50)	1(50)	1(50)
B. cereus	1	1(100)	0(0)	1(100)	0(0)	1(50)	1(100)	0(0)	1(100)	1(100)
Total	**112**	**73(65.2)**	**72(64.3)**	**51(45.5)**	**65(58.04)**	**55(49.1)**	**102(91.07)**	**67(59.8)**	**99(88.39)**	**100(89**

Values in brackets indicate antimicrobial susceptibility in percentage,

TET =tetracycline, CMP =chloramphenicol, S =streptomycin, Oxa =oxacillin, Amp =ampicillin, Su =sulfisoxazole,

ERY =erythromycin, K =kanamycin, CL = clindamycin, n: total number of isolates tested.

4.4.1. Frequently used antibiotics to treat case of mastitis

A one-year retrospective data (2006) obtained from clinical records in the study area showed that the types of antibiotics used to treat mastitis were Pen Strep (penicillin and, streptomycin combinations), oxytetracycline, and intramammary infusion, procaine penicillin, mastitis injector (Table 10).

Table 10: Mastitis cases and type of drugs used for therapy in Gondar veterinary clinic (2006).

Types of drugs	**No. of mastitis cases treated**	**%**
Pen Strep	26	30.95
Ox tetracycline	14	16.67
Intra mammary infusion	26	30.95
Mastitis injector	15	17.86
Procaine penicillin	3	3.57
Total	**84**	**100**

4.4.2. Questionnaire results

Nearly 44.01% of the housing materials used were local materials, 10.56% modern materials (cement) and 45.34% small holders had an open-air system with his animals tied to trees and posts. Floor types were 20.50% concrete, 25.16% arranged stones and 54.35% earth. Stones arranged on the floor were uneven with prominent crevices between the stones where urine, dung and water could easily collect and stagnate. Only 4 farms had maternity pens which have adequate space, dry and well ventilated.

From all the houses 91.93 % of the houses provided adequate space for the animals and over crowding is not a problem. There was a ventilation problem in almost 40% of the houses and some houses are completely sealed, resulting in humid hot conditions inside with the strong smell of ammonia. In addition, 59.63% of the structures had poor drainage systems resulting in the accumulation of urine and faecal matter. This was particularly observed in houses with the stone floors.

An assessment of the cows' cleanliness was also done. Most cows had soiled udders and this was correlated with dirty houses and more having drainage problems. All the dairy holders milked their cows by hand, twice a day and 87.27% reported that they dried their cows abruptly after 7 months of pregnancy. The majority of the small holders said they did not cull a cow because of mastitis, and those that had done so would only cull after the lost of two or more teats. Only 14.29% of the farmers checked milk for abnormalities at each milking. The methods used in most cases were stripping the milk to the floor, in the hand or milking container. All the farmers washed the udder using water from a common bucket for several cows until the water became evidently dirty.

Nearly all-dairy holders were not aware of the importance of segregation and milking mastitic cows test to prevent the spreading of mastitis amongst cows. All the cows were milked inside the barn where thy stayed. None of the farms had a purpose built milking parlor at the time of the study.

None of the dairy holders were aware of dry cow therapy and teat dip as a means of controlling mastitis. Among those who had experienced their cows for mastitis 72.36% consulted a

veterinarian or veterinary assistant. The most commonly used drugs were penicillin_ streptomycin combination, oxytetracycline, intramammary infusion, mastitis injector, and procaine penicillin. The general farm hygiene was 53.11% classified as poor from hygiene standards point of view.

Table 11: Results of the questionnaire survey on housing, environmental management, milk hygiene control measures and treatment procedures (n=322)

		Count	Percentage
	Ventilation		
	- Excellent	82	25.47
	- Satisfactory	114	35.40
	- Poor	126	39.13
	Soiling of udder		
	- In apparent	15	4.66
	- Moderate	179	55.59
	- Serious	128	39.75
	General farm hygiene		
	- Excellent	36	11.18
	- Satisfactory	115	35.71
	- Poor	171	53.11
	Drainage system		
	- Excellent	18	5.59
	- Satisfactory	112	34.78
	- Poor	192	59.63
	Drying off method		
	- Abrupt	281	87.27
	- Intermittent	41	12.73
	Use of teat dip		
	- Yes	0	0
	- No	322	100
	Administration of dry cow therapy		
	- Yes	0	0
	- No	322	100

	Knowledge of teat dipping - Yes - No	 0 322	 0 100
	Method of milk checking - Floor or hand or milking container - CMT - P_H card	 47	 14.60
	Time of milking mastitic cows - First - Last - With out consideration to udder health status	 9 313	 2.80 97.20
	Teat washing - Yes - No	 167 155	 51.86 48.14
.	Teat drying before milking - Yes - No	 89 78	 27.64 24.22
.	Material used for teat drying - Cloth rag - Air - Sponge	 17 72	 5.28 22.36
	Use of separate cloth - Separate cloth rag - Shared cloth rag	 17	 5.28
	Hand washing between each milking - Yes - No	 8 314	 2.48 97.52

	Use of disinfectant for hand washing - Water only - Soap - Disinfectant	 5 3	 1.55 0.33
	Mastitis situation in the farm - Yes - No	 67 255	 20.81 79.19
	Person treating mastitic cows - Veterinarian - Vet. Assistant - Myself	 49 233 40	 15.22 72.36 12.42

5. DISCUSSION

5.1 Prevalence and associated risk factors

This study showed the overall prevalence of mastitis in local and crossbred cows from private smallholder farms investigated cross sectionally. Clinical prevalence accounted at cow level for 0.93% in crossbred and none in local zebu breeds. The result obtained in this study was low when compared with the reports of Bishi (5.3%), Workineh (25.1%) in Addis Ababa, and Gizat (3.9%) in Bahir Dar.

The prevalence of subclinical mastitis in crossbreds at cow level in the present study (34.72%) agrees with the report in Addis Ababa, 34.30%, (Bishi, 1998), Dire Dawa in eastern Ethiopia 38.6%, (Darsema, 1992), Debre Zeit in central Ethiopia 39.5%, (Zerihun, 1996). However, the finding in this study was lower than results reported in Bahir Dar, 44.6%, (Gizat, 2000), Arsi, 53.0%, (Takele, 1987), and Soddo, 45.9%, (Biffa, 1994)

The variability in the prevalence of bovine mastitis is due to interactions of several factors mainly of management, environment and factors relating to animal and causative organisms, This study also showed a wider difference in prevalence of mastitis between localities which could be attributed to the variation in veterinary services, herd management practices in cow handling, nutrition, milking procedures, sanitation and housing as these predispose the individual animal as well as herds to the mastitis.

Sub clinical mastitis in both breeds has been reported to be higher than clinical mastitis. This is due to the defense mechanism of the udder, which reduces the severity of the disease. Higher yielding cows have been found more susceptible to mastitis owing to position of teat, udder and anatomy of teat canal, making them prone to injury and due to fewer efficacies of phagocytic cells in higher yielding cows. In Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Hussein *et al.,* 1997) while the high economic loss could come from subclinical mastitis. Usually Ethiopian dairy farmers' especially small holders are not well informed about the invisible loss from sub clinical mastitis (Hussein *et al.,* 1997). The relatively high prevalence of mastitis in the left rear quarters (26.70%)

and right front quarter (25%) in this study agrees with the finding of others. This may be due to greater production capacity of hindquarters, the likelihood of fecal and environmental contamination, and ease of first grasping by milker's hand in case of right front quarters.

Among the risk factors considered to have effect on the occurrence of mastitis, breed was found to be statistically significant. The odds of occurrence of mastitis were two times more likely in crossbreds compared to local zebu. Therefore, the lower prevalence in local zebu breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and Mc Daniel, 1985). In addition to physical barrier, difference in occurrence of mastitis in these breeds could arise from difference in cellular immunity (Eriskine, 2001).

Breed influence on prevalence of mastitis could be attributed to the difference in certain physiological and anatomical characteristics of the mammary glands, occurrence of mastitis may be influenced by some heritable characteristics such as capacity of milk production, teat structure and udder conformation.

Stage of lactation was found to affect mastitis prevalence significantly. The figure was lower for cows at mid lactation . These results are consistent with previous reports. Absence of dry cow therapy regime could possibly be among the major factor contributing to high prevalence at early lactation. The high incidence of mastitis around calving is largely a consequence of high new infection rate in the dry period and a peri parturient suppression of host defenses. Increasing mastitis with age is probably not due to increased intramammary susceptibility but to increase ease of penetration of the teat duct by pathogens and accumulated previous infections.

This study confirmed that mastitis was also distributed in extensive dairy husbandry practices and becoming a major health problem of indigenous dairy cows. There was no significant difference in prevalence of mastitis between husbandry practices because of absence of variation in hygienic standards of dairy environment and milking conditions, also no significant difference between high and low udder tick load.

The high occurrence of mastitis-induced blind mammary quarters, which has a direct influence on milk production with a subsequent impact on food security, signifies the importance of the problem. Lack of screening and treatment of sub clinical mastitis and inadequate follow up of clinical and chronic cases coupled with persistent challenges of the mammary glands by microbial pathogens could be the main predisposing factors to quarter blindness. This hidden and gradual destruction of the mammary tissues would end with non-functional quarters.

During the day, the animals were allowed to roam in an open field and were housed at night. The benefits of this were that the barn could be adequately cleaned when the animals were outside and the barn would dry adequately before the animals came in for the evening. This must have been an effective way of controlling environmental mastitis pathogens.

In Gondar, dairy farms have been intensified through introduction of high-milk-yielding exotic breeds, and this has increased the supply and consumption of milk and milk products. The fact that all except 12.42% of the dairy holders consulted a veterinarian or a veterinary assistant was an encouraging finding; by this, farmers are able to benefit from expert advice.

The knowledge and use of dry cow therapy, teat dipping, culling, and the use of conventional disinfectant were generally absent from all farms. Many of the farmers were reluctant to cull their cows in chronic cases of mastitis. Some of the respondents only cull a cow after it had lost two quarters of its udder while others would cull a cow only when it had lost all quarters.

Inadequate hygienic condition of dairy environment, poor animal health services, and lack of proper attention to health of the mammary gland were important predisposing factors of mastitis in the area. Adequate housing with proper sanitation and regular screening for early detection and treatment, follow up of chronic cases, culling of older cows with repeated attacks,

Injuries caused by ticks and vigorous suckling by bigger calves are known to cause direct inflammatory reaction to the mammary gland, necrosis and abscess formation, which may lead to udder damage, and/or exposure to serious secondary infections. Therefore, in developing a

mastitis control strategy the role of tick infestation should be given due attention and tick control measures and prompt treatment of teat / udder injuries are recommended to alleviate the problem.

It is essential that systemic records are kept regarding the epidemiology of cow mastitis; status of infections, treatment patterns would provide useful management information to the producer, veterinarian and other mastitis control team members. There is thus a need to routinely investigate and record the epidemiology of bovine mastitis and antibiogram sensitivity of bacterial isolates from the smallholder sector in the study area. Any purchased animals, including heifers and cows must be isolated and cultured before entering the milking herd; such animals may introduce contagious microorganisms.

Post milking teat dipping, routine dry cow therapy reduces the prevalence of CNS in dairy herds. Also, avoidance of teat end injury prevents colonization of the udder by microorganisms. To reduce occurrence of mastitis caused by environmental microorganisms, the cows' environment must be kept as clean and dry as possible. Moisture of any kind, such as rain, humidity, urine, drinking water and even udder wash, favors the growing of environmental microorganisms and should be minimized.

5.2. Bacterial isolation

In this study, S*taphylococcus aureus* and CNS were the major mastitis inducing pathogens detected. CNS was the predominant pathogens involved constituting (31.1%) of all isolates. This finding was at variance from earlier investigations in other regions in Ethiopia. Bishi (1998) and Hussien *et al.* (1997) reported 54% and 42% isolation rates of *S. aureus* and CNS respectively. In Denmark, out of 4645-quarter milk samples examined to determine the distribution of bacterial species in bovine mastitic milk, CNS was the second predominant isolate next to *S. aureus* (Nickerson, 1987). Workineh *et a*l. (2002) reported isolation of CNS at a rate of 2.5% lower than the present study. The high isolation rate of CNS in this study could be associated with lowered resistance of the cow due to teat injury. *Staphylococci* typically colonize a broken skin and hence the risk of colonization and subsequent transfer into the udder increases. Actually, the effect of tick and/lesion on the occurrence of mastitis in this study was not significant.

In the Coagulase negative staphylococci, the primary source of these organisms appears in skin contamination tending to be associated with a lack of teat dipping. Some contribution to the number of these organisms can occur from intra mammary infections, but skin flora appears to be the major factor. The uses of post milking teat dipping and routine dry cow therapy markedly reduce the prevalence of Coagulase negative staphylococci in dairy herds. Cure rate for lactation therapy are usually poor, but dry cow therapy will cure more than 80% of existing infections. Pre dipping with an effective germicide will control new infections. The isolation rate of *S. aureus* (16.5%) in this study was the next to CNS and closely comparable with the findings of Bishi (1998) and Hussien *et al.* (1997) reported 9% and 10.69% prevalence in Addis Ababa, respectively. However, the present finding was lower than that of Workineh *et al.* (2002), Kerro and Tareke (2003) where *S. aureus* accounted for 39.2% and 40.5% of the isolates, at Addis Ababa and Southern Ethiopia respectively, The relatively high prevalence of *S. aureus* in this study could be associated with lack of effective udder washing and drying, post milking teat dip and drying, inter cow hand washing and disinfection in the milking routine of the area, contamination of milkers hands, low culling rate of chronically infected cows and limited knowledge of dairy holders on segregation as a control option has been reported to quickly lead to spread of mastitis and *Str. uberis* (6.7%) were isolated. This finding was higher than the

finding of Kerro and Tareke (2003) who reported isolation rates of 13.1% *Str. agalactiae*, 5.6% *Str. dysgalactiae Streptococci* species were also among the dominant (36.6%) bacterial population as mastitis pathogens in and around Gondar milk shed. *Str.agalactiae* (15.9%), *Str. dysgalactiae (*14.0%) and *Str. uberis* 5.1%.

The spread of *Streptococcus agalactiae* like *Staphylococcus aureus* occurs during milking. Excellent milking hygiene and the use of an effective teat dip could reduce transmission from cow to cow. In this study, contagious pathogens showed greater frequency than others (*S. aureus* being the most common). Poor milking and management practices and hired milkers who milk in more than one farm could explain this. The explanation given for *S. aureus* could also apply for *Str.agalactiae* and *Str. dysgalactiae*.

In this study, environmental pathogens for *Str. uberis*, *Str. dysgalactiae*, and *Escherichia coli* were isolated. There is a common understanding that with increasing herd size, manure disposal and sanitation problems leads to build up of bacterial population (Coli forms and environmental Streptococci) in the cow's immediate environment. In this study, the average herd size was two lactating cows. The number of hours dairy cows kept indoor is also a factor that will increase the possibility of contact of teats with the environmental pathogens.

Environmental mastitis pathogens were found to be the most frequent isolates from clinical quarter cases in a random sample of dairy herds in Southern Netherlands (Miltenburg *et al.,* 1996). In the milking barn, the floor of the udder and teats should be as clean and dry as possible prior to milking. This is extremely important because "wet milking" of cows increases the incidence of infections caused by environmental *Streptococci.* Generally, the key to control is through good sanitation.

Generally, in small holder management system as in this study, that cows were allowed to graze for longer hours a day on the pasture land and supplemented with concentrate and hay when they return home late in the after noon. This might minimize their stay indoor and hence minimal exposure rate to environmental pathogens. The low prevalence of clinical mastitis in this study could be associated with this management system

5.3. Antibiotic susceptibility test

The present study was under taken to determine the resistance pattern of bovine mastitis causal bacteria to commonly used antimicrobials in the study area to provide information to concerned animal health professionals. The antimicrobials response rate may be qualified as poor when it cures less than or equal to 25% and said favorable when the response rate attains 75% or above. The selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability. Tetracycline, Pen Strep, intramammary infusion, mastitis injector was commonly used antimicrobials for the treatment of mastitis in the study area. The selection of the types of antimicrobial agents was made based on clinical considerations including frequent use of the drug in the study area and availability. Tetracycline, Pen strep, intramammary infusion, mastitis injector was commonly used antimicrobials for the treatment of mastitis in the study area.

The poor inhibitory effect of ampicillin against *Staphylococcus aureus* strains observed in this study is in agreement with what was reported by Mackie *et al.* (1988). The latter reported that the *S. aureus* isolates sensitivity to ampicillin was 19 and 17 percent from clinical and subclinical mastitis respectively for the year 1986 in Northern Ireland.

In this study, *S. aureus* isolates were most susceptible to kanamycin, sulfisoxazole, clindamycin, oxacillin, chloramphenicol, erythromycin, streptomycin, and tetracycline. *S. aureus* showed resistance to ampicillin. Bishi (1998) obtained comparable results where erythromycin and oxacillin were effective on *S. aureus* where as streptomycin was less effective where only 18% of the total isolates were susceptible. In the present study, unlike (Bishi, 1998) tetracycline and chloramphenicol were effective only in 12% and 18% of the isolates. *E. coli* was highly resistant to erythromycin and highly susceptible to chloramphenicol and kanamycin. This was lower than the report given by (Eriskine *et al.,* 2001).

In general sulfisoxazole, clindamycin and kanamycin were showed very good efficacy; tetracycline, chloramphenicol, erythromycin, and Oxacillin showed moderate efficacy; whereas streptomycin and ampicillin showed poor efficacy in almost all isolates.

Each herd should have a treatment protocol designed by a consulting veterinarian. A limited selection of drugs, chosen according to the results from bacteriological diagnoses and sensitivity tests should be used for treatment. Treating sub clinical mastitis during lactation with antimicrobial drugs should be avoided due to substantial economic losses arising through milk having to be discarded and unsatisfactory cure rates. Treatment of clinical mastitis should always also include monitoring of therapy results.

The present study demonstrated the existence of alarming level of resistance of frequently isolated mastitis bacteria to commonly used antimicrobial agents from prolonged and indiscriminate usage. It is therefore, very important to implement a systematic application of an in vitro antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra mammary infections. Mean while, due to limited sample size tested in the present study additional studies involving larger sample size and dairy herds will have greater use to formulate guidelines with regard to the choice and use of antibiotics in both the treatment and prevention of intra mammary infections.

6. CONCLUSIONS AND RECOMMENDATIONS

Prevalence of bovine mastitis in smallholder farms in and around Gondar was to be high. This shows that bovine mastitis is an important dairy health problem in the area.

Sub clinical mastitis in both breeds were higher than clinical mastitis, breed, age, parity, and stage of lactation were the risk factors, *Staphylococcus aureus* and Coagulase negative staphylococci were the major mastitis inducing pathogens detected.

It was found that for antimicrobial susceptibility sulfisoxazole, clindamycin, and kanamycin were showed very good efficacy. Tetracycline, chloramphenicol, erythromycin, and oxacillin showed moderate efficacy. Streptomycin and ampicillin showed poor efficacy in the majority of isolates.

Usually Ethiopian dairy holders especially smallholder are not well informed about the invisible loss from sub clinical mastitis, this form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases while the high economic could come from sub clinical mastitis.

Based on the results of the present study, the following recommendations are forwarded:

- In this study, the major isolates were contagious pathogens, therefore; special attention should be given to practice such as udder preparation, teats should be clean and dry before milking, use of single towel for each cow and adequately sanitized between milking. Excellent hygiene and post milking teat dipping must be exercised to prevent new infections, disinfecting hands before milking and between milking, avoidance of fresh cows milking with cows that have clinical mastitis, cows that have had several cases of clinical mastitis, and cows with chronic infections should be culled.

- Good ventilation, adequate manure removal, farm cleanliness and sanitation, good drainage system, maximum air movement through housing, feeding and calving facilities should be provided to reduce the number of environmental micro organisms. Moisture of any kind, such as rain, humidity, urine, drinking water, and even udder wash, favors the growing of environmental microorganisms and should be minimized.

- Ampicillin and streptomycin showed poor efficacy in the majority of the isolates sulfisoxazole, clindamycin and kanamycin were showed very good efficacy, therefore; could be the drug of choice for the study area.

- Creating awareness among the dairy producers about how milking methods and hygiene can help to prevent mastitis.

- Udders hair should be clipped to minimize the amount of manure clinging to the glands, bedding of barn is important to reduce environmental mastitis.

- Cow environment should be as clean and dry possible, should not have access to manure, mud or pools of stagnant water.

- Properly design and maintain free stalls and stanchions, keep calving areas clean, properly bedded (straw preferred).

- Segregate chronic mastitis cows, milk them last, cull when necessary because they serve as reservoirs of organisms and could infect susceptible cows.

- Milking equipment should be adequate in size and regularly cleaned and maintained.

- Examine periodically teats and teat ends.

- To maximize milk production in the region the Bureau of agriculture should introduce systems that increase awareness on sub clinical mastitis to dairy holders.

- The future for smallholder dairy development should rely on continued research and education of small holders themselves

7. REFERENCES

Auldist, M. J., Coats, G. L., Roger, G. M., McDowell, G. H. (1995): Change in the composition of milk from health and mastitic dairy cows during lactation cycle. *Aust.j. of Exp. Agric.* **35**: 427-436.

Biffa D: (1994): The study on the prevalence of bovine mastitis in indigenous zebu cattle and jersey breeds in Wollaita Soddo, characterization and in vitro drug sensitivity of the isolates. D.V.M thesis, Debrezeit: faculty of veterinary medicine, Addis Ababa university: Ethiopia.

Biru, G. (1989): Major bacteria causing bovine mastitis and their sensitivity to common antibiotics. *Eth. J. Agric. Sci*. **11**: 47-54

Bishi, A. B. (1998): Cross-Sectional and Longitudinal Prospective Study of Bovine Clinical, Sub clinical Mastitis in Peri-urban and Urban Dairy Production System in Addis Ababa Region. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit, Ethiopia.

Blood, D. C. and Radostits, O. M. (1989): Mastitis In: Veterinary Medicine, A Text Book of the Disease of Cattle, Sheep, Goat, and Horses, 7th ed. Bailliere Tindal. London. Pp 501-599.

Blowey, R. W. (1990): A Veterinary Book for Dairy Farmers. 2nd ed. Ipswich: Farming Press Ltd. PP: 181-229.

Brooks, B. W., Barnum, D. A., Meek, A. H. (1983): An Observational study of *Corynebacterium bovis* in selected Ontario Dairy Herds .*J. Comp*. *Med*. **47**: 73-78.

Calvinho, L. F, Almeda, R. A., and Oliver, S. P. (1998): Potential virulence factors of *Streptococcus dysgalactiae* associated with bovine mastitis. *Vet. Microbiology*, **61:** 91-110.

Carter, G. R. (1984): Diagnostic Procedures in Veterinary Bacteriology and Mycology. 4th Ed. Ilions, USA: Charles Thomas Publisher.

Darsema G .A survey of bovine mastitis in different dairy farms DireDawa autonomous and East Hararghe administrative region.D.V.M thesis. Faculty of veterinary medicine, Addis Ababa University, Ethiopia.

Dinsmore, R. P., English, P. B. R., Gonzalez, W. (1992): Use of augmented cultural techniques in the diagnosis of the bacterial causes of clinical bovine mastitis. *J. Dairy Sci.* **75**: 2706-2712.

Dohoo, I. R. and Meek, A. M. (1982): Somatic cell counts in bovine milk. C*ana. Vet. J.* **23**: 119-125.

Dorgent-Mellna, P., Searlett, J., Pollock, R. V. H., and Sears, P. (1988): Herd-level risk factors for *Staphylococcus aureus* and *Streptococcus agalactiae* intrammamary infections. *Prev. Vet. Med.* **6**:127-142.

Emanuelson, U. (1997): Use of individual somatic cell count in monitoring herd status. *Live. Prod. Sci.* **48**: 239-246.

Eriskine, R. J. (2001): Mastitis control in dairy herds. In: Herd Health: Food Animal Production Medicine. 3rd Ed, W. B. Sounders Company, Philadelphia. Pp 397-435.

Eriskine, R. J., Eberhart, R. J., Spencer, S. B. and Cambell, M. A. (1988): Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. *J. Am. Med. Assoc.* **192**: 761-765.

FAO, (2003): Livestock Sector Brief. Livestock Information, Sector Analysis and Policy Branch April 2003. 1-15. Rome, Italy.

Faull, W. B. and Hughe, J. W. (1985): Mastitis Notes for the Dairy Practitioners. Liverpool University Press. Pp. 4-7.

Gizat, A. (2004): A Cross-sectional Study of Bovine of Mastitis in and around Bahir-Dar and Antibiotic Resistance Patterns of Major Pathogens. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia..

Hamann, J. (2003): Definition of the physiological cell count threshold based on changes in milk composition. *Bull. of Inter. Dairy Fed.* **381:** 56-78.

Harmon, R. J. and Langloris, B. E. (1988): Prevalence of Minor Pathogens and Associated Somatic Cell Count. In proc. 25th annual management. National. Mastitis Counsel., Columbus, OH. Natl.Mastitis counc, Inc., Ahrligton, VA. 11.

Hillerton, J. E, Morgan W. E., Farnsworth, R., Neijenhuis, F., Bains, J. R., Main, E. A., Bailliere Tindal. London Ohnstand, I., Reineman, D. J. and Timma, L.(2001): Evaluation of bovine teat condition in commercial dairy herds, infectious and non-infections. In: Proceedings of the 2nd International Symposium on Mastitis and Milk Quality, Compton, United Kingdom. Pp 352-356

Hogan, T. S., Smith, K. L., Hoblet, K. H., Schoenberger, P. S., Todhunter, D. A., Hoesten, N. D. (1988): Field survey of clinical mastitis in low somatic cell count herds. *J. Dairy Sci.* **72**: 1547-1556.

Hussien, N., Yehualashet, T., Tilahun, G. (1997): Prevalence of mastitis in different local and exotic breeds of milking cows. *Eth. Jour. Agr. Sci.* **16**: 53-60.

Jabb, K.V. F. and Kennedy, P. C. (1993): Pathology of Domestic Animals. 4th ed. Vol.II., New York, San Francisco, London: Academic Press, INC. Pp: 430-435.

Kassa T., Wirtu, G., Tegegne, A. (1999): Survey of mastitis in dairy herds in the Ethiopian central highlands. *Ethio. J. Sci.,* **22**, 291-301.

Kerro, O. (1997): A study on bovine mastitis in some selected areas of southern Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM Thesis.

Kerro, O. and Tareke, F. (2003): Bovine mastitis in selected areas of southern Ethiopia. *Trop. Animal Heal. and Prod.* **35,** 197-205.

Larsen, D. (2000): Milk quality and mastitis. *Veterinary Microbiology*. **7**1, 89- 101.

Lemma, M., Kassa, T., Tegegene A. (2001): Clinically manifested major health problems of crossbred dairy herds in urban and per urban production systems in the central high lands of Ethiopia. *Trop. Animal Heal. and Prod.* **33**: *85-89.*

Mackie D. P, Logan E. F, Pollock D. A. Rodgers S.P. (1988): Antibiotic sensitivity of bovine Staphylococcal and Coliform Mastitis isolates over four years. *Vet. Rec*, **123**: 515_517.

Marthe, E. H. and Ellckson, B. E. (1959): Problems created by the presence of antibiotics in milk and milk products. *J. Milk Food Tech*. **22**: 296-272

Miltenburg, J. D., Lange, D., Crauwels, De. , Bongers, A. P. P.,Tielen, and M. J.M., Schunkcen, Y. M., Elbers, A. R. (1996): Incidence of clinical mastitis in a random sample of dairy herds in the Southern Netherlands. *Veter. Rec.* **139**: 204 – 207.

MOA. (2004): Budgeting and planning reports, summary of MOA., North Gondar zone, 1987-88:1, Pp 14-20.

Mohammed, A. M., Ahmed, Simeon., Ehui and Yemesrach, Assefa., (2004): Dairy development in Ethiopia. *Ethio .j. Sci* **32**: 10-22.

Mungube, E. O. (2001): Management and economics of dairy cow mastitis in the urban and peri urban areas of Addis Ababa (Addis Ababa milk shed). Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, MSc Thesis.

Nelson, W. P., and Stephen, C.N., (1991): mastitis counters attack. Babson Bros. Co. 1880 country Farm Drive Naperville, Illinois 60563, U.S.A. 60563 Pp.18- 59.

NMC (1990): Microbiological procedures for the diagnosis of bovine udder infection. 3rd ed. Arlington VA: National mastitis council Inc.

Nesru, H. (1999): A Cross- Sectional and Longitudinal Study of Bovine Mastitis in Urban and Peri Urban Dairy System in the Addis Ababa Region Msc, Thesis.Free university of Berlin and Addis Ababa University, Ethiopia.

Nickerson, S. C. (1987): Resistance mechanisms of the bovine udder: New implications for mastitis control at the teat end. *J. Am Vet. Med. Assoc*. **91**: 1484 -1494.

Oudessa, K. (1997): A Study on Bovine Mastitis in Some Selected Areas of Southern Ethiopia. DVM Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit, Ethiopia.

Owens, W. E., Ray, E. H., Watts, J. L., and Yancey, R .T. (1997): Comparison of success and results of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. *J. Dairy Sci.* **80**: 313-317.

Pearson, J. K. and Mackie, D. P. (1979): Factors associated with occurrence, cause, and outcome of clinical mastitis in dairy cattle. *Veter. Rec.* **105**: 456 – 463.

Perry. B. D, Carter F. W., Hill, M. E., G., Milne,A. C. (1987): Mastitis and milk production in cattle in a communal land of Zimbabwe. *British Veterinary Journal.* **143**: 44-50.

Quinn, P. J., Carter, M. E., Morley, B., Carter, G. R. (1994): California Mastitis Test (CMT). In: Clinical Veterinary Microbiology. 1st ed. Wolfe publishing, London. Pp 333-334.

Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (1999): Mastitis. In:Clinical Veterinary Microbiology, Mosby International Limited, London. Pp 327-344.

Radostits, O. M., Blood, D. C. and Gay, C. C. (1994a.): Mastitis. In: Veterinary Medicine: Text Book of the Diseases of Cattle, Sheep, Goat and Horses. 8th. ed. Baillier Tindal, London. Pp 563-614.

Radostits, O.M., Leslie, K. E, and Fetrow, W. J.(1994b): Mastitis control in dairy herds. In: Herd Health; Food Animal Production Medicine. 2nded., W. B. Sounders Company Philadelphia. Pp 229-276.

Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchllif, K. W (2000): Mastitis. In: Veterinary Medicine. 9th ed., Harcourt. Ltd, London. Pp 603-700.

Rainard, P and Peutrel, B. (1988): Effect of naturally occurring intra-mammary infections by minor pathogens on new infections by major pathogens in cattle. *Am. J. Vet. Res*. **49**: 327.

Ryser, E. T and Marthe, E. H. (1989): “New” food borne pathogens of public health significance. *I am diet Assoc*.**89**: 948_954

Sargeant, T. M., Scott, M. M., Leslie, K. E., Ireland, H. K., Bashiri, M. J. A. (1998): Clinical mastitis in dairy Cattle in Ontario: Frequency of occurrence and bacteriological isolates. *Can, Vet.J*. **39**: 33-38.

Schalm, D. W., Caroll, E. J., Jain, N. C. (1971): Bovine Mastitis. Lea and Febiger. Philadelphia. Pp 182-282.

Sears, P. M., Gonzalez, R. l. N., Wilson, D. Z., Han, H. R. (1993): Procedures for mastitis diagnosis and control. In, Hunt, E. (Ed.), The Veterinary Clinics of North America, Food Animal Practice, Update On Bovine Mastitis. W. B. Saunders Company. Pp. 445-456.

Seykora, A. J. and McDaniel, B.T. (1985): Udder and teat morphology related to mastitis resistance. *J. Dairy Sci*. **68**: 2087-2093.

Sol, J., Sampimon, O.C., Snaep, J. J., and Schkken, Y.M. (1997): Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis cauesd by *S. auerus. J. Dairy Sci*.**80**: 2803-2808..

Takele S. (1987) A study of the prevalence of bovine mastitis in different dairy farms in, Arsi region. D.V.M thesis. faculty of veterinary medicine, Addis Ababa University, Ethiopia.

Tefera, G.(2001): Prevalence of mastitis at Alemaya University Dairy Farm, *J. Eth. Vet. Assoc*. **17**: 21.

Tesfu. K, Gezahegn, W. and Azage, T. (1999): Survey of mastitis in dairy herds in the Ethiopian Central Highlands. *Ethi. J. ag. Sci.* **22**: 291 – 301

Torgerson, P. R., Eibbs, H. A., Anderson, D.B. (1992): High incidence of clinical mastitis due to *staphylococcus aureus* in two dairy herds with low milk cell counts. *Vet. Rec*. **130**: 54-55.

Tegegene, A., and Yimegnuhal, A., (2004): Milk production of Adaa Liban woreda and dairy products marketing assertion. Newsletter No.10 Ethiopia society of animal production (ESAP). Addis Ababa, Ethiopia.

Thrusfield, M. (1995): Veterinary epidemiology. 2nd ed. Blackwell Science. Pp 182-190

Watts, T. L., and Salmon, R. A. (1997): Activity of selected antimicrobial agents against strains of *staphylococcus aureus* isolated from bovine intra mammary infections that produce b-lactamase. *J. Dairy Sci.* **80**: 788-791.

Watts, T. L. (1988): Etiologic agents of bovine mastitis. *Vet. Microbial.* **16**: 41-46.

WHO, (1999): Future trends in veterinary public health. Report of a joint WHO/FAO/OIE expert committee on veterinary public health, Teramo, Italy.

Wilson, D. J. Gonzalez, R. N., Das, H. H. (1986): Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *J. Dairy Sci.* **80**: 2592-2598.

Winkller, J.K., (1982): farm animal health and disease control. Pp.152 – 155

Workineh, S., Molla. B., Hailemariam, M. and Potgieter, L. N. D (2002): Prevalence and etiology of mastitis in cow from two major Ethiopian Dairies Farms. *Trop. Anim. Hlth and prod*. **34**: 19-25.

Zerihun T. (1996) A study on bovine sub clinical mastitis around Debrezeit. DVM thesis Debrezeit faculty of veterinary medicine, Addis Ababa University: Ethiopia

8. ANNEXES

Annex 1. Survey format for Mastitis problem herd

- Owner's Name ....Address .... Telephone ....

I. Cow History

- Cow Id .... or Local Name...
- Breed .... Age .... Parity ....Stage of lactation..........................
- Previous exposure to mastitis: Exposed ....Not exposed ....
- Udder tick load: Low.................Moderate.............High..
- Husbandry system: Extensive................ Semi intensive...
- Teat lesion: Present................ Absent ....
- Gross milk quality : Watery.............. Bloody................. Clots/Flakes .... Normal.
- Is the general condition of animal acceptable? Yes .... No………… ....
- Are laboratory services utilized? Yes .... No ....
- Are breeding records kept? Yes .... No ....
- Clinical cases per month ....
-Where are most clinical cases ? Dry cow .... Lactating cows ...... Both ....
- Number of cows culled for mastitis in previous years ? .... … . .
- Source of replacement raise .... purchase ....
- Sample collected from RR .... RF .... LF .... LR ....
- CMT score RR .... RF ....LF ....LR ....

General comments…………………………………………………………………………..

……………………………………………………………………………………………

II. Housing

- Building material used: Modern....... Local........ Mixed ....Out door...........................
- Housing type: Barns.............. Open pen .... Out door..
- Floor type: Concrete............ Arranged stone .... Earth..
- Does the sunshine enter barn ? Yes .... No ....
- Types of bedding: None .... Sawdust .... Shaving .... Straws ....
- Corrugated iron roof ? Yes .... No ....
- Wall: Concrete............... Mud............ Other ....

- Separate maternity pen? Yes .... No ....
- Maternity pens dry and well bedded ? Yes .... No ....
- Adequate space for the cows? Yes .... No ....
- Manure removal: Daily...........Weekly.........monthly..............Other (specify)................
- Drainage system : Excellent .... Satisfactory .... Poor ....
- Condition of housing/bedding : Excellent .... Good .... Bad ....

Generalcomments...
....

III. Milking procedure

- Do you give exercise to the animals? Yes .... No ....
- How are teats and udders washed? None....... Common cloth rag .... individual cloth towel...
- How are teats and udders dried? None .... common cloth rag .... individual cloth towel .... Air.......... Sponge ....
- Are udders clean prior to milking ? Good .... Fair .... Poor …………
- Is fore milk examined regularly for abnormalities ? Yes .... No.........................
- What method do you use for checking? Floor or hand or milking container .... CMT .... pH card ....
- Do most cows milk out within 3 to 5 minutes ? Yes .... No ....
- Are cows milked out adequately ? Yes .... No ....
- Are cows over milked ? Yes .... No ....
- When do you milk cows with mastitis? First .... Last .... With out consideration to udder health status..
- Do you practice milking mastitis cows last? Yes .... No ....
- Is feed provided after milking to encourage cows to stand for at least 1 hour ? Yes........No ....
- Do you wash your hands between each cow ? Yes .... No ....
- What do you use for washing your hands ? water only .... soap .... ………..........
disinfectant.... ………………………………………………………………………
- Overall rating: Excellent .... adequate .... Fair .... Poor.... ....

Generalcomments...

....

IV. Drug Usage

- Is mastitis a problem in your herd? Yes .... No ....
- Who advises on products to use for treatment? Veterinarian .... Vet. assistant ……………..
......…..............Animal health. technician.................... Yourself …………………………………
- Which drugs do you use for treating mastitis?

....

- Who treats the animal? Veterinarian .... Vet. assistant ....Animal health technician............. Yourself...
- Used for preparation for intramammary treatment? Antiseptic ....Soap .... Water only ......Nothing ....
- Are cows treated gently? Yes .... No ....
- Was the treatment successful ? Yes .... No ....

Generalcomments...
....

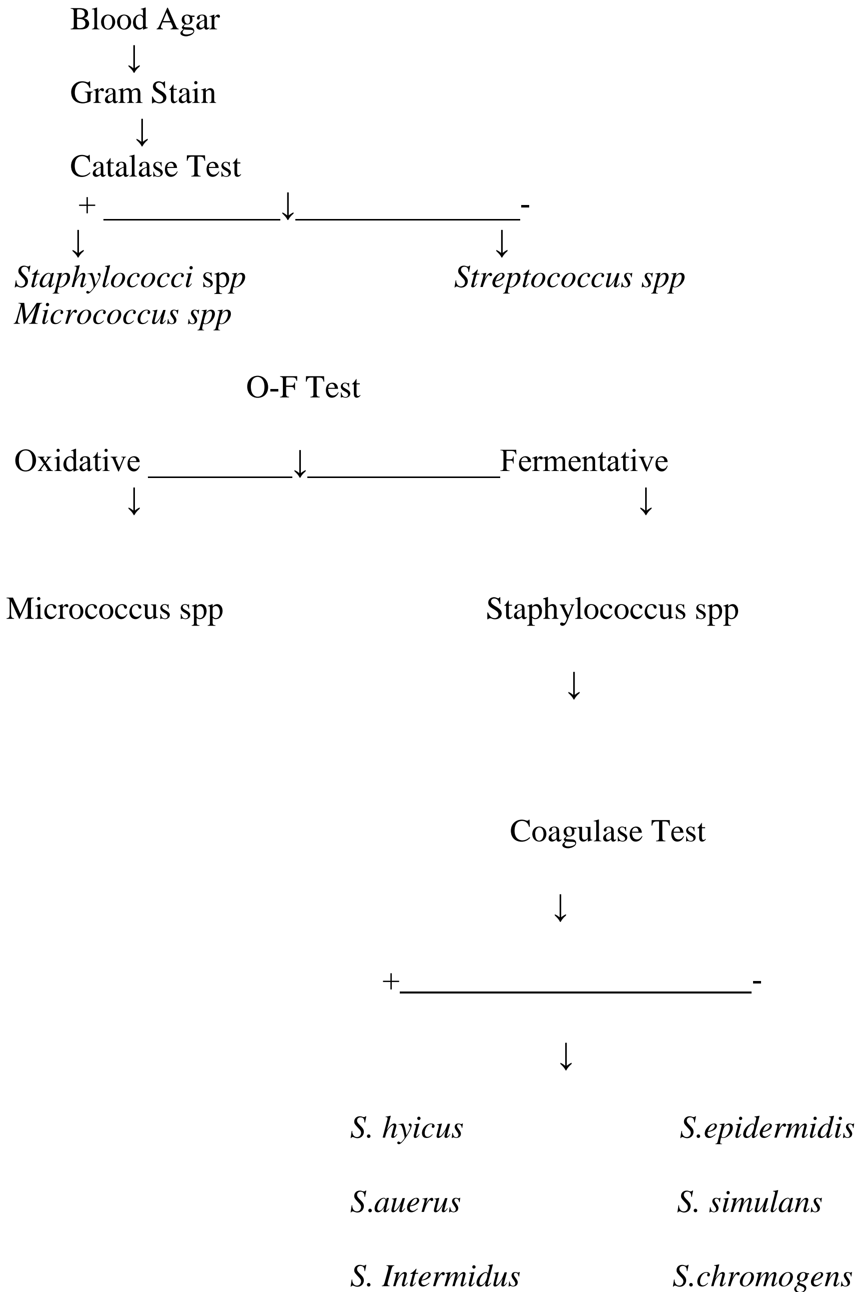
Annex 2. Interpretation of CMT findings (Quuin *et al.*, 1994; Schalm *et al.*, 1974).

Score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
T(Trace)	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickens with gel formation
3	Strong positive	Viscosity greatly increased strong gel i.e. cohesive with a convex surface

Annex 3. Procedures for the identification of mastitis pathogens (Carter, 1984; Sears *et al.,* 1993; Quinn *et al.*,1994)

I. Gram positive cocci

A. Differentiation of Mastitis Causing Staphylococcus spp



II) Gram Negative Rods

Species	Oxidase	MacConkey (Lactose Fermentation)
Pseudomonas spp	+	
Pasteurella spp	+	
E. coli	-	+
Klebsiella spp	-	+
Enterobacter spp	-	+
Serratia spp	-	-
Proteus spp	-	-
Citrobacter spp	-	-

Annex 4. Procedures to conduct antibiotic susceptibility test (Carter, 1984)**.**

- Preparation of the inoculums

Inoculation of a distinct colony into 5ml of nutrient broth and incubated at 35-37^{0}C for about 5 hours. Then the turbidity was compared with 0.5MacFarnand standard. This standard was prepared by adding 0.5 ml of 1 %(11.75g/litre) $Bacl_{2.}2H_20$to 99.5ml of 1 % (0.36N) $H_2SO_{4.}$

- Inoculation onto Mueller-Hinton Agar

Mueller-Hinton Agar cooled to 50^{0}C and poured into a sterile Petri dish on a level surface to a depth of 4 mm. This was equivalent to 60ml in a 15cm plate and about 25 ml in a 10 cm plate. For slow growing bacteria 5% defibrinated whole blood was added. Then a sterile cotton swab on a wooden applicator stick was used to transfer the diluted bacterial suspension to a plate; excess fluid was squeezed out by rotating the swab against the sides

of the tube. The plate was seeded uniformly by rubbing the swab against the entire agar surface in three different planes roughly 60 degrees to each other.

- Disc Application

Within 15 minutes (time used to dry the inoculums) after the plates were inoculated, antibiotic impregnated discs were applied to the surface of the inoculated plates by hand using a sterile forceps. All discs gently pressed down on to the agar with forceps to ensure complete contact with the agar surface. The discs were no closer than 1.5 cm to the edge of the plate and 3 cm apart from each other. The large Petri dishes easily accommodated 9 discs in outer ring and three in the center (10 cm plates).

- Incubation

Incubated the plates inverted aerobically for 16 to 18 hours at 35^0C.

- Interpretation

Inhibition zone was measured in millimeters using a transparent ruler on the under surface of the Petri dish. For measuring purpose the end point was taken as complete inhibition of growth as determined by naked eye. The result was interpreted according to the table presented below.

Zone Size Interpretive Chart for Antimicrobials

Inhibition Zone Diameter (mm)

Antimicrobial agent	Disc potency	Resistance	Intermediate	susceptible
Streptomycin S10	10 µg	≥11	12-14	≤15
Tetracycline TE30	30 µg	≥14	15-18	≤19
Erythromycin E15	15 µg	≥13	14-17	≤18
Chloramphenicol C30	30 µg	≥12	13-17	≤18
Oxacillin	1 µg	≥17	18-24	≤25
Kanamycin K30	30 µg	≥13	14-17	≤18
Sulfisoxazole	25 µg	≥12	13 - 16	≤17
Ampicillin(AM10)Gram-negative rods and enterococci	10 µg	≥11	12-13	≤14
Ampicillin(AM10) Staphylococci and highly penicillin-sensitive organisms	10 µg	≥20	21-28	≤29
Clindamycin	2 µg	≥15	16-20	≤21

Source: (Carter, 1984)

Annex 5. Primary identification tests

Gram's stain (Carter,1984)

Procedure:

- A thin smear was made.
- Allowed the film to dry in air.
- Fixed the film by passing through the Bunsen flame several times.
- Flooded the slide with crystal violet for 60 seconds.
- Poured off the stain and washed the remaining stain with iodine solution
- Washed off the iodine and shake the excess water from the slide.
- Decolorized with acetone alcohol.
- Counter stain with safranin for 60 seconds and washed with water.
- Observed under a microscope with x100 magnification.

Catalase test (Quinn *et al.,* 1999)

Principle: The break down of hydrogen peroxide into oxygen and water is mediated by the enzyme catalase.

Procedure: A loopful of the bacterial growth was taken from the top of the colonies avoided the blood agar medium. The bacterial cells were placed on a clean microscope slide and a drop of 3% H_2O_2 was added. An effervescence of oxygen gas, within a few seconds, indicated a positive reaction.

Oxidase test (Quinn *et al.,* 1999)

Principle: The cytochrome oxidase enzyme is able to oxidize the substrate tetra methyl-p-phenylenediamine dihydrochloride, forming a coloured end product, indophenols.

Procedure; prepared a solution of 1 % tetramethyl-p-phenylenediamine dihydrochloride, then a piece of filter paper was moistened in a petri dish with fresh reagent and the test bacterium was

streaked firmly across the filter paper with a glass rod. A dark purple colour along the streak line with in 10 seconds indicated a positive reaction.

O-F test (Quinn *et al.,* 1999)

Procedure: Prepared O-F base medium and when the O-F base had cooled to 50 ^{o}C added 20 ml of sterile glucose solution into 200 ml of O-F base, for a final concentration of 1% glucose and dispensed into tubes. Two tubes of the O-F medium heated in a beaker of boiling water immediately before use to drive off any dissolved oxygen and the tubes were then cooled rapidly under cold running water. Both tubes were stab-inoculated with the bacterium and a layer of sterile paraffin oil was layered on top of one of the tubes (sealed tube) to a depth of about 1 cm and the tubes were incubated at 37°C and examined in 24 hours and then daily for up to 14 days.

Motility test (Quinn *et al.,*1999)

Procedure: SIM medium (BBL) was used to detect motility and the medium was stab inoculated using a straight wire. Then the tube was examined for motility after 24 and 48 hours. If there is a diffuse growth throughout the medium, the bacterium is motile. The growth of a non-motile bacterium was confined to stab line. To interpret the results, hold the tube against a good light and compared the inoculated tubes with uninoculated one.

CAMP test (Quinn *et al.,* 1999)

Procedure: A culture of the *Staphylococcus aureus*, with a wide zone of partial haemolysis (beta haemolysin) was streaked across the center of a sheep blood agar plate. A streak of the suspected Group B *Streptococcus* was made at right angles to, and taken to with in 1 to 1.5 mm of *Staphylococcal* streak. The plate was incubated at 37°C for 18- 24 hours. A positive CAMP test was indicated by an arrow- head of complete haemolysis. The group B *Streptoccocci* produces a diffusible metabolite that completes the lyses of the red cells, only partially haemolysed by the beta- haemolysin of the *Staphylococcus.*

Annex 6. Secondary identification tests

Indole test (Quinn *et al.,* 1999)

Principle; indole positive bacteria possess an enzyme tryptophanase which converts tryptophan to indole.
Procedure; Stab inoculated SIM medium with test bacterium and incubated at 37° C for 18 to 24 hour. Then added kovacs reagent (0.2ml) to tube and stand for 10 minutes.
Interpretation; the formation of dark red ring indicates positive reaction while in negative reaction a yellow ring is formed.

Methyl red (MR) test (Quinn *et al.,* 1999)

Principle: It is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic, and formic)
Procedure: Inoculated MR-VP broth with pure culture of test organism and incubated at 37°C for two days, then added 5 drops of MR solution into the media.
Interpretation: production of red color indicated a positive result and yellow colour negative in methyl red test.

Voges – Proskauer (VP) test (Quinn *et al.,*1999)

Principle: some organisms produce acetone as the chief end product of glucose metabolism and form less quantity of mixed acids.
Procedure: Inoculate MR-VP broth with pure culture of the test organism and incubate at 37° C for 2 days. Then aliquot 1 ml of broth to a clean test tube and add. 0.6 ml of 5% α-naphthol followed by 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes.
Intepretation: A pink colour indicates a positive reaction.

Urease test (Quinn *et al.,* 1999)

Principle: Urease is an enzyme possessed by many species of microorganism that can hydrolyze urea with the formation of ammonia (alkaline).

Producedure: The surface of the agar slant was streaked with the test organism and incubated at 37^{o}_{C} for 18 to 24 hours.

Interpretation: Organisms that hydrolyze urea rapidly produced positive reaction with in 1 or 2 hours. Red (pink) colour through out medium indicated positive reaction.

Annex7. Differentiation of mastitis causing *Staphylococcus* and *Micrococcus* spp.

Test	*S. aureus*	CNS	*Micrococcus*
Catalase	+	+	+
Coagulase	+	-	-
Haemolysis	+	-	-
Mannitol (A)	+	-	-
Maltose (A)	+	V	-
Glucose (A)	+	+	-

+= Positive reaction, -= Negative reaction, V= variable reaction, A= acid production
CNS=Coagulase negative staphylococci

Annex 8. Differentiation of mastitis causing *Streptococcus* spp.

Species	CAMP Test	Growth on MacConkey	Easculin hydrolysis	Other Confirmatory Tests
Str. agalactiae	+	-	-	Mannitol + (A)
Str. uberis	$\pm$	-	+	Salicin + (A)
Str. dysgalactiae	-	-	-	Salicin+, Mannitol +
Str. faecalis	-	+	+	Salicin (-)
Str. pyogenes	-	-	-	Mannitol (-)
Str. pnuemoniae	-	+	+	Mannitol (-)

+= Positive reaction, -= Negative reaction, $\pm$ = positive or Negative

Annex 9. Differential test used for *Bacillus* spp.

Bacillus spp.	Citrate	Arabinose	Mannitol	Voges Proskauer
B.stearothermophilus	-	V	-	-
B. cereus	+	-	-	+
B. pumilus	+	+	+	+

+= Positive reaction, -= Negative reaction V= variable reaction

Annex 10. Differential tests used for Corynebacterium &Actinomyces spp

Corynebacterium and *A.pyogenes*	Catalase test	Haemolysis	Glucose	Lactose	Maltose	Trehalose
C. ulcerans	+	V	+	-	+	+
C.bovis	+	-	-	-	-	-
C.pseudotuberculosis	+	+	+	+	+	+
A. pyogenes	-	+	+	+	+	V

V=Variable réaction,
Source: Carter, (1984), Quinn *et al*. (1999)

Annex 11. Differential test used for Gram- negative rods

G-ve bacteria	1	2	3	4	5	6	7	8	9
E.coli	+	+	-	-	+	-	Gas+,H_2S^-	-	+
K.pneumoniae	-	-	+	+	+	+	Gas+,H_2S^-	-	+
P.aeuroginosa	-	-	-	+	-	-	$^-$Gas+,H_2S^-	+	+
p.mirablis	-	+	-	+	-	+	Gas^+,H_2S^+	-	+
p. multocida	+	-	-	-	-	-	H_2S^+	+	-

G-ve=Gram positive

1= indole test, 2= Methyl red test, 3= Voges Proskauer test, 4= Citrate utilization, 5= lysine decarboxylase test, 6= urease test, 7= TSI test, 8= oxidase test, 9= Growth on MacConkey agar

Annex 12. Media used for isolation and identification of bacteria.

Blood Agar Base (BBL), Becton Dickinson, USA)

Composition (g/1): Heart muscle, infusion from (solids) 2.0; pancreatic digest of casein13.0; Yeast extract 5.0; Sodium Chloride 5.0; agar 15.0
Preparation: Suspend 40.0g of the powder in 1 liter of distilled water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 Minute to completely dissolve the powder. Autoclaved at 121^0C for 15 minutes. Cooled the base to 50^0 C and added 5% sterile defibrinated blood.

MacConkey Agar (Oxoid, Hampshire, England)

Composition (g/1): peptone 20.0 lactose 10.0, bile salts No.31.5 sodium chloride 5.0; neutral red 0.03; crystal violet 0.001agar 15.0
Preparation: Suspend 51.5g in 1 liter of distilled Water. Bring to boil completely. Sterilize by autoclaving at $121^o{}_C$ for 15 minutes.

Nutrient agar (Oxoid, Hampshire, England)

Composition (g/1):"Lab-Lemco" powder 1.0yeast extracts 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0.
Preparation: Suspend 28_g in 1 liter of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121^0 C for 15 minutes.

Edwards medium, modified (Oxoid ,Hampshire, England)

Composition (g/1): "Lab-Lemco" powder 10.0; peptone 10.0; aesculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallus sulphate 0.33; agar 15.0.
Preparation: suspend 41_g in 1 liter of distilled water. Bring to the boil to dissolve completely. Steriliz by autoclaving at $115^0{}_C$ for 20 minutes. Cool to 50^0C; add 5 to 7% of sterile sheep blood. Mix well and pour plates.

9. CURRICULUM VITAE

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SIGNED DECLARATION SHEET

“This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged “

Name NIBRET MOGES

Signature ________________

Date of submission________________

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

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Dr. Kelay Belihu ___________________