ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF VETERINARY MEDICINE

CROSS-SECTIONAL STUDY OF BOVINE MASTITIS IN URBAN AND PERI-URBAN DAIRY FARMS OF HARAR TOWN

BY

KETEMA BOGALE GELETE

JUNE 2010
DEBRE ZEIT, ETHIOPIA

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Tropical Veterinary Epidemiology

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LIST OF ABBREVATIONS

CAMP	Cristine Alkine Munch Peterson
Cm	Centimeter
CMT	California Mastitis Test
CNS	Coagulase Negative *Staphylococcus*
FAO	Food and Agricultural Organization
g/l	gram per liter
IMI	Intra Mammary Infection
IU	International Unit
KOH	Potassium Hydroxide
LF	Left Front
LH	Left Hind
m^2	meter square
µg	Microgram
mm	Millimeter
MR	Methyl Red
NCCLS	National Committee for Clinical Laboratory Standard
O-F	Oxidation Fermentation
PCR	Polymerase Chain Reaction
RF	Right Front
RH	Right Hind
SCC	Somatic Cell Count
SIM	Semi solid motility medium
TSI	Triple Sugar Iron agar
USA	United States of America
USD	United States Dollar
VP	Vogues Proskaures

ABSTRACT

A cross sectional study of bovine mastitis was carried out from September 2009 to May 2010 in urban and peri-urban dairy farms of Harar town to determine the prevalence of mastitis on cow and quarter level, to isolate and identify etiological agents for mastitis and to assess the effect of associated risk factors. From 36 dairy farms 341 lactating cows were investigated. Of these 143 cows (41.9%) were positive by California Mastitis Test (CMT) and 138 cows (40.5%) were culture positive for mastitis. Among culture positive cows 23 (6.8%) and 115 (33.7%) were infected clinically and sub clinically, respectively. Out of 1364 quarters 51 were blind, 228 (17.4%) were positive by CMT and 214 (16.3%) by culture. Frequency of clinical and sub clinical mastitis on quarters were 41 (3.1%) and 173 (13.2%), respectively. Logistic regression analysis showed that prevalence of mastitis was significantly higher in third parity than the first ($p<0.05$) in early lactation stage than in late ($p<0.05$). Hygiene, ventilation and drainage were also found to be significantly associated with the occurrence of mastitis ($p<0.05$). However, herd (farm) size and quarters position were not significantly associated with mastitis ($p>0.05$). From the isolated mastitis causing pathogens *Staphylococcus* species were the most prevalent (42.9%) followed by *Streptococcus* species (28.3%), *Enterobacter* species "Coliforms" (20.0%) and other gram positive bacteria (8.8%). Through the questionnaire survey it was confirmed that poor farm management, unhygienic milking practices, lack of knowledge about the role of risk factors on mastitis occurrence and hidden (subclinical) mastitis are reasons for the increased prevalence of mastitis (40.5%) in the study area. Antimicrobial susceptibility test showed that most of the isolates in this study were found to be highly sensitive to cloxacillin, gentamycin and amoxicillin, and moderately sensitive to ampicillin and oxytetracycline. However, *Staphylococcus* and *Streptococcus* species were highly resistant to streptomycin and penicillin.

Key words: milking cow, mastitis, prevalence, questionnaire, risk factor, udder, quarter, CMT, culture, sensitivity test, dairy farms, Harare

1. INTRODUCTION

Mastitis, inflammation of the mammary gland, is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the dairy industry. Mastitis is a management related disease whose prevention and control depends among other factors on the type of management employed (Mungube *et al.,* 2004). Mastitis is a result of a combined interplay of exposure to microbes, cow defense mechanisms and environmental and managemental risk factors (Suriyasathaporn *et al.,* 2000). The causes of mastitis are almost entirely infectious and mostly bacterial, but may also be caused by non-bacterial pathogens and chemical or physical agents (Wallenberg *et al.*, 2002). Mastitis causing organisms can be excreted through the udder directly into the milk or may originate from the skin and mucous membranes of the animal or milker and environment (Bradley, 2002). Mastitis can be classified as contagious and environmental. Contagious mastitis is caused by bacteria, which reside primarily in the mammary gland and spread from cow to cow during milking (Erskine, 2001). Environmental mastitis is associated with microorganism, present in the environment (Quinn *et al.,* 2002). Mastitis can be clinical or sub clinical type. The clinical type is manifested by signs, which vary with the severity of the disease and generally include pain, heat and swelling of the affected quarter or half of the gland and abnormality of milk either as clots or flakes and wateriness of the liquid phase. Sub clinical type is usually not observable and it can be detected by direct or indirect Somatic Cell Count (SCC) methods, which are essentially measurements of the concentration of leucocytes in the milk (Mifflin, 2004).

Mastitis is recognized world wide as a major cause of economic loss due to reduced milk production, discarding of mastitic milk and following antibiotic therapy, increased labor, veterinary and drug cost, early culling of cows, replacement cost and death of per acute cases (Mifflin, 2004).

Ethiopia holds a large potential for dairy development. The country currently manages the largest livestock population estimated at about 48 million cattle (CSA, 2008). The total annual milk production is estimated at 797,900 to 1,197,500 metric tones of raw milk, out of which between

85% and 89% is derived from cattle. Per capita availability was estimated at 17 to 18 kg per year compared to the global average of 100 kg/year (FAO, 2003). Different constraints can be mentioned as cause of the low level of milk production in Ethiopia, mastitis being one of these.

Mastitis has received very attention in Ethiopia on which many reports are made by (Bishi 1998; Nesru 1999; Lema *et al.* 2001; Workineh *et al.* 2002; Kerro Dego and Tareke 2003; Mungube *et al.* 2004, 2005; Getahun 2006 and Nibret 2007), and others. However, most of the studies were carried out in Addis Ababa, the surrounding, central highlands of Ethiopia and in other limited southern and north west areas of the country.

To the researcher's knowledge in Harari Peoples National Regional State, mastitis is not a disease that is well considered, despite its economic and public health importance. In line with this assessing new area and obtaining information on disease prevalence and associated risk factors may contribute for designing and implementing effective prevention and control strategies.

Therefore, the objectives of the present study include:

- To determine the prevalence of bovine mastitis
- To isolate and identify etiological agents of bovine mastitis in the study area
- To identify risk factors associated with mastitis

2. LITERATURE REVIEW

2.1. General definition of bovine mastitis

Inflammation of the mammary gland caused by infectious and non-infectious agents and mostly bacteria that invade the udder, multiply and produce toxin that are harmful to the mammary gland (Mifflin, 2004).

Clinical mastitis: is expressed by the production of abnormal milk flakes, clots or watery secretions from the infected quarter showing clinical signs such as, swelling, heat and pain on palpation. In case of acute clinical mastitis, these signs may be combined with general signs such as hyperthermia, anorexia, depressed general condition (Gruent, 2001).

Sub-clinical mastitis: causes decreased milk production and lower milk quality and contagious bacteria may spread to other quarters. Nevertheless, as often go untreated during the lactation sub clinical mastitis does not pose threat to the cow's life or its ability to produce the main sign is an increased somatic cell count (SCC) which leads to financial losses to the farmer (Gruent, 2001).

2.2. Etiology

More than 130 microbial species have been isolated from bovine mastitis milk samples (Quinn *et al.*, 2002). The microbial cause of mastitis include a wide variety of bacteria (aerobic, facultativly anaerobic, microaerobic and anaerobic), yeasts, fungi, moulds, algae and virus (Deluyker, 2005). Among microorganisms that cause infection in mammary gland, the most common causes are designated in to two categories. Contagious pathogens - that colonize the mammary gland of cows. The source of infection for contagious pathogens is usually an infected mammary gland and can be spread by milking machines milker's hands and towels. It includes *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, Corynebacterium bovis and* CNS which are commensally persist in the mammary gland (Quinn *et al.*, 2002).

Environmental pathogens- that do not normally infect the mammary gland, but can do so when the cows' environment the teats and udder, milkers hands or the milking machine are contaminated with these organisms and they gain access to the teat cistern. Environmental Pathogen includes *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogens, Yersinea, Serratia sp, Proteus sp, Bacillus sp, Enterococcus faecalis Pseudomonas sp.* CNS, environmental *streptococci, Corynobacterium,* Yeast or Fungi and many other (Quinn *et al.*, 2002).

Streptococcus disgalactiae appeared to occupy an intermediate position between the contagious and environmental groups of pathogens. It can be found in the environment and has been isolated from the tonsil, mouth and vagina of cows (Quinn *et al.*, (2005).

Etiological agents of mastitis can be grouped in to major and minor pathogens based on the frequency of isolation and their involvement on the occurrence of mastitis (Quinn *et al.*, 2002).

2.2.1. Major Pathogens of Bovine Mastitis

Major pathogens are those microbial organisms involved more frequently on the occurrence of bovine mastitis. More commonly, they are *Staphylococcus aureus, Streptococci agalactiae, Mycoplasma bovis, Escherchia coli, Klebsiella, Streptococcus disgalactiae, Streptococci uberis and Corynebacterium pyogens* (Marion, 2007).

2.2.2. Minor Pathogens of Bovine Mastitis

Minor pathogens are those organisms infrequently associate with bovine mastitis and are CNS, *Actynomyces bovis, Bacilus cerus, Crynebacterium ulcerans,* environmental *Streptococci, Proteus, Pasteurella sp., Yersinia, Nocardia* and other (Quinn *et al.*, 2005).

2.3. Epidemiology

2.3.1. Risk Factors

Agent factors

The microbial factors which affect mastitis occurrence have the ability to survive in the immediate environment of the animal. The ability to colonize the teat duct, the ability to adhere to mammary epithelium and not be flushed out with the milk flow, the degree of invasiveness, the ability to resist phagocytosis and antibacterial substances in the udder, including resistance to antibiotics (Quinn *et al.,* 1994).

Host factors

Stage of lactation is a potential determinant of both sub clinical and clinical mastitis. Mastitis due to environmental organisms is most common in the first few days after calving and regresses as lactation progresses. This is due to the reduction in the immune function soon after calving (Radostits *et al.,* 1996). Parity has been shown to a risk factor for both sub clinical and clinical mastitis. Young animals have a decreased susceptibility through more effective host defense mechanisms (Dulin *et al.,* 1998). Nutrition and per parturient diseases such as dystocia, parturient paresis, retained placenta has been identified as risk factors for subsequent development of mastitis (Radostits, 2001). Genetic variation in conformation of the udder teats, sphincter tone and anatomy of the teat canal, determined in part by heredity, can be considered as component of genetic resistance variation among cows (Radostits, 2001).

Environmental factors

Dairy and milking management have been shown to be essentially important risk factors. Milking machine malfunction or inadequate design, milking shade environment including poor hygiene predispose cows to mastitis. Coliform mastitis is more common in housed cattle than those at pasture are. Herd size is another factor associated with the incidence of clinical mastitis. As herd

size, increases manure disposal and sanitation problems increases exposure to potential pathogens in the immediate environment of the animal (Radostits, 2001).

2.3.2. Mastitis in Ethiopia

In order to indicate the prevalence, incidence and distribution of mastitis and mastitis causing agents, a number of epidemiological studies were carried out in different areas of Ethiopia. Among the studies Bishi (1998) reported an over all prevalence of sub clinical and clinical mastitis of 30.2% and 5.5%, respectively in urban and peri-urban dairy production system in and around Addis Ababa. In another studies a prevalence of 39% and 37.2% on cows level was reported by Abdella (1996) and Nesru (1999) respectively. In a study conducted at Repi and Debre-zeit dairy farms, out of 186 lactating cows, 21.5% and 38% were clinical and sub clinically infected, respectively (Workineh *et al.*, 2002).

A study carried out on bovine mastitis in selected areas of southern Ethiopia revealed an overall prevalence of 58.3%, clinical 37% and subclinical 62.9% in Holstein fresian cows (Kerro and Tareke, 2003). Mungube *et al.* (2004) indicated overall prevalence of subclinical mastitis 46.6% at cow levelin central highlands of Ethiopia. Recently Demelash *et al.* (2005) confirmed 36.9% prevalence with 16.11% clinical and 34.6% sub clinical mastitis in lactating dairy cows in southern Ethiopia. Getahun (2006) in small holder dairy farms of central highlands of Ethiopia identified a prevalence of clinical and sub clinical mastitis of 2.6% and 22.3% respectively. Similarly, Tamrat (2006) reported a prevalence of 23.4% and 54.3% clinical and sub clinical mastitis, respectively, in Sodo area and Nibret (2007) reported a prevalence of 34.7% at cow level.

The most common mastitis causing agents isolated during the studies were *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus hycus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus disgalactiae, Streptococcus faecalis, Escherchia coli, Klebsiella, Actinomyses bovis, Corynebacterium pyogenes, Proteus, Micrococcus species* and *Bacillus cer*eus.

Table 1: Summary of studies on the prevalence of mastitis in Ethiopia

Area	Prevalence			Source
	Clinical (%)	sub clinical (%)	Both (%)	
Wondogent	-	-	39	Abdella *et al.* (1996)
Urban peri urban Addis Ababa	5.5	30.2	-	Bishi (1998)
Urban peri urban Addis Ababa			37.2	Nesru (1999
Debere-Zeit dairy farms	21.5	38	-	Workeneh, *et al.* (2002)
Southern Ethiopia	37	62.9	58.3	Kerro and Tareke (2003)
Cental highlands of Ethiopia		46.6		Mungube *et al.* (2004)
Southern Ethiopia	16.11	34.6	36.9	Demelash *et al.* (2005)
Cental highlands of Ethiopia	2.6	22.3	-	Getachun (2006)
Wolayta Sodo	23.4	54.3	-	Tamerat {2006)
Around Gonder			34.7	Nibret (2007)

2.3.3. Zoonotic significance

In most places in Ethiopia milk is consumed raw, this poses a risk to the public as pathogenic bacteria are acquired by consuming raw milk. Milk allows the growth and multiplication of a number of potentially harmful microorganisms. It is a vehicle for the transmission of many zoonotic diseases. The major infectious diseases transmitted through milk are tuberculosis, brucellosis, typhoid, paratyphoid, dysentery, staphylococcal intoxication, and diphtheria (Heeschen, 1994). Enterotoxigenic *staphylococcus aureus* is a cause of food poisoning in humans characterized by vomiting, headache, abdominal pain and diarrhea that occur 1-6 hours following consumption of contaminated milk (Marthengo and Ombui, 2007). Human listeriosis can be resulted from consumption of contaminated milk (Quinn *et al.*, 2005).

2.3.4. Economic importance

Economic losses due to mastitis are recognized world wide as a major problem on dairy farms. Financial loss involved as a result of permanent loss of production in individual cows, discarded milk following antibiotic therapy, early culling of cows, veterinary costs, drug cost, increased labor, death of per acute cases, replacement costs and high leukocyte count that lead to a loss income if a bonus scheme is operating for milk with low cell count are losses caused by the disease (Mifflin, 2004).

Average costs of clinical mastitis cases can range form 100 to 200 USD/case/year and 40 to 100 USD/herd/year (Radostits, 2001). Bishi (1998) also reported that the economic losses from clinical and subclinical mastitis in Addis Ababa milk shed to be approximately 270 Ethiopian birr per lactation. Mungube (2001) estimated the economic losses from mastitis in the urban and peri urban areas of Addis Ababa, losses due to mastitis (milk production lose, treatment cost, withdrawal losses and culling losses) were estimated to 210.8 birr per cow per lactation from which milk production loss contributed 38.4%.. In another study conducted in the same study area by Mungube (2005) was reported that a quarter with mastitis, loss an average of 17.2% of its milk production.

2.3.5. Pathogenesis

Infection of intramammary gland always occurs via teat canal. Nevertheless, the development of mastitis is more complex. The most well clarified stages are invasion, infection and inflammation. Following invasion, a bacterial population may be established in teat canal, which multiplies rapidly and extended into mammary gland tissue where infection occurred frequently or occasionally depending on its susceptibility (Radostits *et al.*, 2000). The potentiality for invasion is greatly increased by bacteria that reside in or colonize the teat duct. Such colonization occurs in both lactating and dry cows, and they may survive for months, serving as source of bacteria for infecting the gland (Schroeder, 1997).

Staphylococcal mastitis

Gangrenous mastitis is caused by the action of the alpha toxin that damages blood vesicles, resulting in ischemic coagulative necrosis of adjacent tissue. The affected quarter becomes purplish cold and will eventually slough, if the animal survives the toxemia. Both clinical and subclinical mastitis lead to a gradual replacement of secretary tissue with fibrous tissue and a subsequent loss of milk production by the affected quarter. The chronic and subclinical forms of mastitis respond poorly to antibiotics because of the development of a tissue barrier that prevent penetration of antibiotics to the site of infection. Dry cow therapy is more likely to be successful but even with this method it is estimated that only 70% of the persistent staphylococcal infection respond to therapy (Quinn *et al.*, 2005).

Streptococcal mastitis

Streptococcus agalactiae resides in the milk and on the surface of milk channels but does not invade the tissue. There is rapid multiplication of the bacterium with a great out pouring of neutrophils in to the ducts with damage to the ductal and acinar epithelium. Ducts are obstructed with cells and debris, causing involution of the acini in the affected lobules. Fibrosis of inter alveolar tissue occurs, which also lead to a loss of secretary function (Quinn *et al.*, 2005).

Coliform mastitis

Escherichia coli and other coliforms can multiply rapidly in the quarters with a low cell count. This elicates an inflammatory reaction that destroys a large proportion of a bacterial population. When gram negative bacteria die and lyse, endotoxin is released from the cell wall. This massive and sudden libration of endotoxin results in a severe, life-threatening toxemia. A unique feature of coliform infection is that, in the cows that recover, the udder tissue gradually returns to normal with out fibrosis. In subsequent lactations, the gland produces to its optimal capacity (Quinn *et al.*, 2005).

Summer mastitis

The syndrome is seen most commonly in Europe. It is a dual infection of *Actynomyces pyogens* and an anaerobic, often *Peptostreptococcus indolicus*. Summer mastitis occurs in non-lactating heifers and cow at pasture in the summer months and tends to the more commen during wet weather. It is thought to be fly born. A massive invasion of the mammary tissue via the teat canal results in a large proportion if a gland being affected at one time. There is a severe systematic reaction and loss of function of the entire quarter. The secretion from the affected quarter is foul smelling, attributable to the activities of the anaerobic *Peptostreptococcu*. If the cow survives, the quarter becomes extremely indurate and abscesses develop, later rupturing through the floor of the udder, often at the base of a teat (Quinn *et al.*, 2005).

2.4. Diagnosis

Clinical examination: Cows with clinical mastitis can be readily identified by visible change in milk composition and physical examination of the udder (Bansan *et al.*, 2005). Clinical examination of mastitis based on clinical signs, e.g. swelling of udder tender to touch, fever and depression. Proper examination requires the use of strip cup preferably one that a shinny, black plate permitting the detection of discoloration as well as clots, flakes and pus. In this method, milk is drawn on the plate in pools and comparison made between the milk of the different quarters (Radostits *et al.*, 2000).

2.4.1. Somatic Cell Count (SCC)

Once bacteria reach the teat canal and progress to milk producing tissue of the udder, the cow's body reacts by sending larger numbers of white blood cell or somatic cells into the mammary gland. This local population of somatic cells includes neutrophils, macrophages and lymphocytes, which serve as one of the most important defense mechanisms that the cow has to fight infection. Somatic cell count is recognized as useful procedure to assess milk quality and udder health (Phillips, 1996). The common somatic cell counting methods are direct microscopic count, in this method a volume of 0.01 ml of milk is spread over a microscope slide, defatted and then stained by methylene blue stain. The leukocytes are counted in 50 fields in the collaborated microscope and the leukocyte number is expressed per ml of milk (Minna, 2004). Electronic SCC of suspicious milk by using coulter milk cell counter, which counts particles as they flow through an electric field, and the somatic milk cell counter, which stains cells with a fluorescent dye and then counts the number of fluorescing particles. In direct microscopy, leukocytes can be counted directly (Quinn *et al.*, 2002).

2.4.2. Strip Cup Test

The simplest way to detect abnormalities in the milk is to use a strip cup which has a black plate onto which milk is drawn from each quarter. Any changes in color or consistency are easily detected. Milk from each quarter must be examined as more than one quarter may be affected. Abnormal milk may show discoloration, flakes, shreds, clots and wateriness (Nelson and Stephan, 1991).

2.4.3. California Mastitis Test (CMT)

CMT is a simple, semi-quantitative test for determining the number of nucleated cells (neutrophils, macrophages, lymphocytes, and epithelial cells) in the milk. A black plastic paddle with four receptacles into which milk is drawn from respective teat is used. In the test, the CMT reagent (14 % Teepol (alkylarylsulphate)) solution is added in an equal quantity onto the milk in each cup. The milk and the reagent are gently mixed by circular motion. The degree of precipitation or gel formation reflects the amount of cells present in the milk (Quinn *et al.*, 2002).

2.4.4. pH Determination

The pH of normal milk may vary between 6.5 and 6.8. In mastitis as lactose production decreases and alkaline salts from the blood enter the milk, the milk becomes more alkaline. Thus, increasing alkalinity of the milk is characteristics of a progressive mastitis condition. Mastitic milk when drawn from the teat may on rare occasion be acidic and is yellow with Bromocresol purple. The pH should be determined on freshly drawn milk, although milk held at refrigerator temperatures for 24 to 28 hours may be used (Coles, 1986).

2.4.5. Chloride Test

The chloride test is dependent upon the determination of an abnormal quantity of chloride in the milk. Normally milk contains 0.008 to 0.14% chlorides. Abnormal milk contains a greater quantity of chloride because of the presence of inflammatory exudates. These exudates contain a considerable quantity of chloride and even a small amount of exudates in milk, which will result in a positive chloride test. The test is conducted by adding 5ml of silver nitrate solutions to 1ml of milk, followed by the addition of two drops of potassium chromate solutions and mixing by inversion of the tubes. The appearance of yellow color indicates that more than 0.14 percent chlorides are present in the sample and a brownish red color indicates that the sample contains less than that amount (Coles, 1986).

2.4.6. Catalase Test

The catalase content of normal milk is low, except at the beginning and end of lactation. The free oxygen gas is trapped in the closed end of a smooth fermentation tube or other device; 5ml of 1% H_2O_2 is mixed with 15ml of milk in the tube and incubated for 3 hours at a room temperature or 37^0C. The volume of gas that accumulates is measured and expressed in volume percent. Normal milk will generally yield less than 10% gas (Morrison *et al.*, 1991).

2.4.7. White Side Test

Place five drops of milk on a glass plate with a dark background or on a Bakelite sheet. Two drops of 4% sodium hydroxide solution are added to the milk and the mixture stirred rapidly with an applicator stick for 20 to 25 seconds. Milk from normal animals will have no change after the addition of the test reagent. Milk from a cow suffering with acute or sub acute mastitis will become thick and viscid, while milk from an animal with a chronic case may have only a few white flakes. Accurate interpretations in terms of the reaction can be made only by comparing the test results with a standard (Coles, 1986).

2.4.8. Electrical Conductivity

Mastitic milk has a higher electrical conductivity than normal milk. This is due to tissue damage and the subsequent increase in sodium and chloride ions in milk. The changes in electrical conductivity are one of the earliest manifestations associated with new infections making the early detection and recording of possible mastitis cases routine. Hand held conductivity meters are also available and may be useful for routine pre milking screening of animals (Savasan, 2002).

2.4.9. Polymerase Chain Reaction (PCR)

The multiplex PCR assay can be used as an alternative method in routine diagnosis for rapid, sensitive, and specific simultaneous detection of *Staphylococcus aureus, Streptococcus agalactie, Streptococcus dysgalactiae*, and *Streptococcus uberis* in milk samples (Phuekets *et al.*, 2002).

2.4.10. Bacteriological Examination

The diagnosis of mastitis (clinical or sub-clinical mastitis) often requires bacteriological examination in order to give specific advice on prevention, and sometimes to achieve effective treatment.

Milk sample collection

Strict aseptic procedures are used when collecting of milk samples in order to prevent contamination with microorganisms present on the skin of cows flasks, udder and teats, on the hands of the samplers and in the barn environment udders and especially teats have to be clean and dry before sample collection. Collected samples should be transported in an ice box, and stored at 4%^{0}C until it will be cultured (Quinn *et al.*, 1999).

Culture

Milk culture of positive quarters, high SCC, chronic infection of the entire herd will also provide a wealth of information concerning udder health and provides a basis for attacking the problem. Combining data from milk cultures and SCC information provides with a herd inventory of mastitis pathogens, a picture of their distribution and an indication of the relative importance of each pathogen within the herd. This information can be used to identify risk factors and critical control points for control program development (Dwight and Yuan, 2002). Sub-culture of the bacterial colony type (s) considered most significant should be made to obtain pure cultures for use in identification tests Once a pure culture is obtained, the results from a few comparatively

simple tests can often identify the bacterium to genus level. A gram-stained smear from the culture will establish the gram reaction (gram positive or gram negative) along with the cellular morphology (Ellen *et al.*, 1994).

Biochemical tests

Once the bacteria have been identified to a genus level, further tests can be carried out to identify the species. Pure culture of a single colony type from blood agar will be transferred to nutrient agar slants from which a series of biochemical tests, which aid final identification of various pathogens, can be done following standard methods (Quinn *et al.*, 2002).

2.5. Treatment

The antimicrobials selected for treatment are usually based on in vitro sensitivity patterns and because of a high degree of resistance to labeled mastitis therapeutic regimes, typically therapy resorts to extra label use (Quinn *et al.*, 1999). Antibiotics used in the treatment of mastitis can be administered by potential or intramammary routes (Quinn *et al.*, 2002). The treatment of intramammary concern anti-microbial substance, which delivered intracisternally into the udder through the teat canal, they are essentially targeted at the treatment of mastitis (Gruent, 2001). Intramammary antibiotic preparations are readily available to farmers in many countries and this easy access is likely to result in excessive antimicrobial chemotherapy (Quinn *et al.*, 2002). Chemotherapeutic agents given by the parental route for the treatment of mastitis should ideally, have certain characteristics such as low minimal inhibitory concentration for pathogen causing mastitis (Edward *et al.*, 2002).

Administration of intramammary antibiotics at the beginning of the dry period is used for the treatment of mastitis caused by contagious pathogens, particularly *Staphylococcus aureus* (Katholm, 2003). Treatment of subclinical cases of mastitis due to environmental organisms such as *Streptococcus uberis*, which are detected late in lactation, may defer until the dry period (Quinn *et al.*, 2002). Antibiotic therapy of drying off is an important means of controlling bovine mastitis because antibiotics may be administered by parentral route as there in no concern about

withdrawal period and residue in milk (Gruent, 2001). Treatment protocol for mastitis should be established for herds and cases including recurrences, clinical outcome of each cases including days of discarded milk and culling, bacterial culture of milk collected from affected quarters, and sound use of pharmacokinetic principles, particularly for drugs selected for parentral administration, are all needed to ensure the most cost effective plan (Radostits, 2001).

2.6. Prevention and control

2.6.1. Contagious Mastitis

Teat dipping

Teat dipping with germicidal solutions immediately after every milking has proved to be an effective milking management practice to reduce the rate of new intramammary infections. Teat dipping is a simple, effective and economical means to reduce bacterial population on teat skin. An effective teat dip, correctly used can reduce the incidence of new udder infections by 50% to 90%. Teat dipping must be done correctly and constantly. In summary, post milking teat sanitization is an extremely important component of good milking management with the objective of killing mastitis pathogens residing on teat skin and thereby reducing up the rate of new intramammary infections (Radositits, 2001).

Pre-milking hygiene and udder preparation

The objective of pre-milking udder preparation and teat sanitation is to reduce the microbial population at the teat end. The aim is to minimize the probability of new intramammary infection (IMI) and have good milking performance (Radostits, 2001). Alternative recommended producers for pre milking udder preparation include water hose washing and manual drying of teat, washing teats with a paper towel in sanitized solution plus drying with a single service paper towel, and using pre milking teat dipping in germicide plus paper towel drying. It is argued that manual teat washing improves stimulation and the release of oxytocin for milk let down. The

overall objective of pre milking sanitation is to have clean and dry teats ready for milking (Radostits, 2001).

Milking order

In herds with significant prevalence of contagious pathogens, such as *Staphylococcus aureus*, establishing a specific milking order may be helpful to limit the rate of new infections. In general, first lactation heifers and fresh cows should be milked first. Cows with a high SCC, chronic clinical mastitis, and current clinical cases should be milked last. The maintenance and management of both SCC and clinical mastitis records become important to make milking order programs work (Radostits, 2001).

Milking machine

The milking machine can influence new IMI rates in several ways: it may be a carrier of mastitis pathogens from one cow to the next; it may serve as a path way of cross infection with in cows. Malfunctioning or improperly used equipment may fail to relieve congestion in teat tissue, with eventual teat end damage and IMI occurring, and abrupt loss of milking vacuum may create changes in air movement of sufficient force to move pathogens past the streak canal defense. Therefore, milking machine must perform properly and consistently. The installation and maintenance of the milking system is done according to approved guidelines (Radosstits, 2001).

Dry cow management and treatment

The objective of udder health management during the dry period is to have a few infected quarters as possible at calving. Thus, infections present at the time of drying off should be eliminated, and the rate of new IMI during the dry period must be minimized. Although the focus of mastitis control schemes for the dry period is often on contagious pathogens, environmental bacteria also need to be considered. Thus the prevention of new dry period infection with environmental agents is essential. Antimicrobial therapy of dry cows at the end of lactation was proposed to eliminate existing infections. Dry cow therapy has become the most effective and

widely used mastitis control method for dairy cows. Its efficacy and advantages are well known. Dry cow therapy also reduces the incidence of new IMI by 50% to 75% (Radostitis, 2001).

Culling

Culling is the most effective method of decreasing the prevalence of chronically infected cows (Radostitis, 2001). The ability to cull infected cows is often limited by the need to maintain ample marketed milk for farm cash flow. Culling must be done judiciously with respect to production, breeding performance, genetic potential and other health problems. Excessive culling to achieve quality milk premiums might have a negative net financial impact on herds. An alternative to culling chronic cows is to chemically dry off an infected quarter (Radostits, 2001).

2.6.2. Environmental mastitis

Reduce exposure

The reservoir of infection is the environment of the cow, which causes a wide variety of organisms as causative agents. Mastitis management practices are successful due to control of environmental mastitis. With respect to environmental mastitis, the goal of every dairy farm should be to expose the teat of a cow to a few bacteria as possible. Dairy producers should provide adequate shade to prevent crowding in warm periods of the year, and if portable shading is used, frequent movement about the pasture is recommended. Dry cow housing and particularly maternity areas should receive attention, particularly, for lactating cows adequate free stall space should be provided. Sand bedding used in well-designed free stalls should be considered as the standard for housing. Proper management and disposal of manure and waste products are also areas of consideration for reducing exposure to environmental mastitis causing pathogens (Radostitis, 2001).

Enhance resistance

Antioxidant supplementation plays a critical role in mammary immune resistance. Selenium and vitamin E supplementation enhances neutrophil killing of phagocytized bacteria. Cows supplemented during the dry period with vitamin E and administered a single injection of selenium before calving had decreased incidence of clinical mastitis (37% reduction) and shorter duration of infection (62% reduction) than un supplemented controls. A decrease in clinical mastitis (57%) and duration of infection (40%) during first lactation was observed in selenium and vitamin E supplemented heifers compared with un supplemented controls (Radostits, 2001).

One of the more promising methods of enhancing mammary resistance for *coliform* (and all grams negative IMI is by using vaccination. A study demonstrated that cows receiving two vaccines (bacterin derived from J5 mutant *E. coli*) doses during the dry period and one dose after calving had an incidence of clinical coliform mastitis of 2.6%, as compared with 12.8% in unvaccinated controls (Gonzalez *et al.*, 1989). A second field trial determined that vaccinated cows had a three fold decrease in clinical coliform mastitis (Cullor, 1991).

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted in Harar town, the capital city of Harari Peoples National Regional State. Harar town is located at 525 kms east of Addis Ababa. The total coverage of the town is about 1740 km^2, while the altitude ranges between 1600 to 1920 meters above sea level. The average annual rainfall ranges from 600 mm to 800 mm and the daily minimum and maximum temperature range are from 17^0C to 20°C and 28°C to 30°C, respectively HRARDB (2009).

3.2. Study Population

The target animals sampled were milking cows within urban and peri-urban dairy farms of Harar town. Total cattle populations in the farms were 1020, and target animals sampled were milking cows within urban and peri-urban dairy farms of Harar town. The farms own Holstein cross breed cows with different level of exotic blood. Very few cows are pure exotic breed Holstein type. Breeding service for cows was carried out by artificial insemination provided by the agricultural office and private bulls rented for the purpose. The study farms were categorized to small, medium and large with number of lactating cows 2-5, 6-15 and more than 15, respectively (Table 2 and 3).

Table 2: Farm distribution in urban and peri-urban areas of Harar town

No	District	Kebele	No of farms	Urban			Peri-urban		
				Small	Medium	Large	Small	Medium	Large
1	Jenela	15	7	3	3	1	-	-	-
2	Hakim	13	1	-	1	-	-	-	-
		16	5	1	-	4	-	-	-
		17	7	2	3	-	1	-	1
3	Hamaresa	18	6	3	-	2	1	-	-
		19	4	1	1	-	2	-	-
4	Sofi	Sofi	1	-	-	-	-	-	1
		Gelmeshira	5	-	-	-	1	4	-
	Total	8	36	10	8	7	5	4	2

Note: Number of farms in category: Large – 9, Medium – 12, Small - 15

Table 3: Cattle population in a study dairy farms

	District	Cows	Heifers	Bulls	Oxen	Calve	Total
1	Jenela	92	13	1	3	19	128
2	Hakim	382	32	6	19	56	495
3	Hamaresa	192	19	4	10	46	271
4	Sofi	99	8	-	5	14	126
Total		765	72	11	37	135	1020

Note: Among 765 dairy cows, 666 were milking during the study period

3.3. Sampling Methods and Strategy

All milking cows found in the 36 dairy farms (herds) constituted the study population from which study animals were randomly selected. The selection of cows to be sampled was made as such a way that each sample unit in all farms had an equal probability of being selected. The sampling frame is the whole list of the lactating cows in all farms. Each number was written on a piece of paper in a hat. Then 341 cowswere selected blindly corresponding to the requird sample size.

For the sample size determination 5% precision at 95% CI and 33.3% expected prevalence from the previous study (Darsema, 1997) in Dire Dawa town (which is the nearest to the study area) were considered.

Therefore, according to Thrusfield (2005), the formula used for the calculation of the sample size was:

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

Where

n = required sample size

CI = 95% confidence interval

P = expected prevalence

d = level of precision

The sample size hence was 341 milking cows.

3.4. Study Design

3.4.1. Questionnaire Survey

Questionnaire survey was conducted to obtain appropriate information about the farm condition and to evaluate risk factors on the occurrence of mastitis. The questionnaire was completed in the study period and queries included were: herd description and farm structure, general production information, health problem, social structure, milking procedure and management, farm inspection and feed and water.

3.4.2. Clinical Examination of Cows

Clinical examination was carried out by observing visible signs of inflammation on the udder and physical changes on milk. The amounts of non-functional (blind) teats were also determined.

3.4.3. Cross Sectional Study

In the farms (herds), overall prevalence and prevalence of clinical and sub clinical mastitis at cow and quarter level were studied by using clinical observations, the result of California Mastitis Test (CMT) of samples from all functional quarters, and results of microbiological examinations of CMT positive samples.

Prevalence of mastitis in general, clinical and sub clinical mastitis at cow and quarter level were calculated as:

$$\text{Prevalence} = \frac{\text{Number of cows or quarter with mastitis}}{\text{Total number of lactating cows or tested quarters}}$$

Risk factors for mastitis considered were parity, stage of lactation, herd size, hygiene, ventilation, drainage and quarter position (front and hind).

Parity 1 represents those cows which calved up to 2, parity 2 cows which calved 3-4 times and parity 3 cows which calved 5 times or more. Stage of lactation 1 represents cows with 1 month of lactation, 2 represents cows with in 2-3 months of lactation, 3 cows with in 4-6 months of lactation and 4 cows, which were with in more than 6 month of lactation.

3.4.4. Screening Test

California Mastitis Test

The test was applied as screening test for selection of samples to be cultured for cow's udder study. The test was carried out immediately before milking, after stimulation of the cow and having discarded the foremilk. A small amount of milk sample drawn from respective teat was placed in to a plastic paddle that has 4 shallow cups, an equal amount of test reagents solution was added on to the milk in each cup. The paddle was rotated to mix the milk and the reagent.

Then, the result of the reaction was scored as 0 (negative), trace (suspicious), 1 (+, weak positive), 2 (++, distinct positive) and 3 (+++, strong positive) depending on gel formation which reflects the amount of somatic cells in the milk (Quinn *et al.*, 2002) (Table 4).

Table 4: Interpretation of CMT result

Score	Visible reaction (gelling)	Interpretation
0	None (milk fluid and normal)	Negative
Trace (T)	Slight precipitation	Suspicious
1, (+)	Distinct precipitation but no gel formation	Weak positive
2, (++)	Mixture thickness with a gel formation	Distinct positive
3, (+++)	Viscosity greatly increased strong gel that is cohesive with a convex surface	Strong positive

3.4.5. Milk Sample Collection

Procedure for the collection of milk samples was conducted according to Quinn *et al.* (1999). Strict aseptic procedures were used in order to prevent the contamination of milk samples with microorganisms present on the skin of cow's udder and teat, the hands of the sampler and in the environment. Teats were cleaned and dried before sample collection. For milk sample collection sterile glass and disposable plastic vials with tight fitting push-on caps were applied. During sample collection, the first few streams of milk were removed and discarded. Each teat ends were scrubbed vigorously with cotton moistened with 70% ethyl alcohol. After samples have been collected CMT positive milk samples were transported in an ice box and stored at 4^0C until it was cultured. Clinical cases of mastitis were diagnosed when visible signs of inflammation in the udder or when changes were observed in the secretion.

3.4.6. Culture

Culturing technique was included primary and secondary plating (inoculation) on to 7% sheep blood agar and MacConkey agar and incubation at 37^0C for 24 to 48 hours. The speed of growth, colony morphology and hemolytic characteristics were recorded. Gram stain from the growth was observed under the microscope in order to identify cellular morphology. Primary and secondary biochemical tests for further identification of isolated organisms to species level was conducted next.

3.4.7. Antimicrobial Sensitivity Test

Invitro antibiotic sensitivity test (Kirby-Bauer disk diffusion method was carried out in order to identify the most effective drugs for mastitis treatment in the study farms. A loop full of colony from the growth of isolates was transferred to the nutrient broth in tubes and incubated at 37 ^{0}C for five hours. Mueller Hinton agar which was used as plating medium was inoculated with the broth (bacterial suspension) by using cotton swab.

Then, antibiotic impregnated paper discs (Oxoid, United Kingdom) were applied and pressed down on to the plates with forceps. Plates were incubated inverted at 35 ^{0}C for 18 hours. The diameter of zones of growth inhibition were measured in millimeters and interpreted as sensitive, intermediately sensitive and resistant according to National Committee for Clinical Laboratory Standard (NCCLS) (Quinn *et al.*, (1999). The drugs used were oxytetracycline, streptomycin, ampicillin, penicillin, cloxacillin, amoxacillin and gentamycin. The number and types of tested isolates were *Staphylococcus aureus* 40, *Streptococcus agalactiae* 20, *Escherchia coli* 15, *Klebsiella* 8, *Corynrbacterium pyogens* 7, *Staphylococcus intermedius* 6, *Streptococcus uberis* 6, *Proteus* 5 and *Corynebacterium bovis* 4.

3.5. Data management and analysis

Data was entered into a data base management software Microsoft Excel computer program. Descriptive statistics and Chi square (χ^2) was applied to work out prevalence of mastitis, proportions of clinical and sub clinical mastitis at cow and quarter level, and to determine an association between the disease and risk factors, respectively. Univariable logistic regression analysis was applied to find out P-value, Odds ratio and 95% confidence interval. Descriptive and analytical statistics were worked out using SPSS (version 15.0) software programs.

4. RESULT

4.1. Prevalence Study

In this study a total of 1364 quarters from 341 milking cows were investigated. Among 1364 quarters 1313 (96.3%) were functional and the rest 51 (3.7%) were blind (not functional). Out of the blind teats 37 and 7 animals had single and double blind teats, respectively.

From the total number of sampled lactating cows, 143 (41.9%) were positive by CMT. Out of CMT positive animals, 138 (40.5%) were culturally positive for mastitis casual agents. Therefore in the studied dairy farms, the overall prevalence of mastitis was 40.5% (Table 5).

Among the tested 1313 quarters, 228 (17.3%) were positive for CMT and 214 (16.3%) positive for by culture. According to the test result right front (RF), right hind (RH), Left front (LF) and left hind (LH), were 15.7, 17.9, 16.2 and 19.5 percent positive for CMT and 14.3, 16.8, 14.8 and 18.8 percent positive for culture, respectively. There was no statistically significant difference among the affected quarters (p=0.258). However, the number of affected hindquarters 119 (18.0%) were higher in number than 95 (14.6%) the front quarters (Table 6).

Table 5: Prevalence of mastitis based on CMT and culture result

Farm (herd) size	No. of farms	No. of cows	CMT		Culture	
			Positive	%	Positive	%
Large	9	225	104	46.2	99	44.0
Medium	12	79	29	36.7	29	36.7
Small	15	37	10	27.0	10	27.0
Total	36	341	143	138	41.9	40.5

4.1.1. Prevalence of clinical mastitis

Twenty-three (6.8%) cows were clinically infected and 41 (3.1%) quarters were positive for mastitis causing agents. Seventeen (73.9%) of clinically positive cows were from large size dairy herds (Table 6).

4.1.2. Prevalence of sub clinical mastitis

Hundred and fifteen (33.7%) cows were infected with sub clinical mastitis. From quarters of these cows 181 (13.8%) and 173 (13.2%) were positive for CMT and mastitis causing agents, respectively. Among the cows with subclinical mastitis 84 cows (73.0%) and 124 quarters (71.7%) were from large herds.

Table 6: Prevalence of clinical and subclinical mastitis on farm, cow and quarter level using culture result

Farm (herd) size	No of herds	No of cows	Cow				Quarter				Total	
			Clinical		Subclinical		Clinical		Subclinical			
			No	%	No	%	No	%	No	%	No	%
Large	9	225	17	7.6	84	37.3	31	3.6	124	14.3	101	44.9
Medium	12	79	4	5.1	19	24.1	6	2.0	31	10.3	23	29.1
Small	15	37	2	5.4	12	32.4	4	2.8	18	12.4	14	37.8
Total	36	341	23	6.8	115	33.7	41	3.1	173	13.2	138	40.5

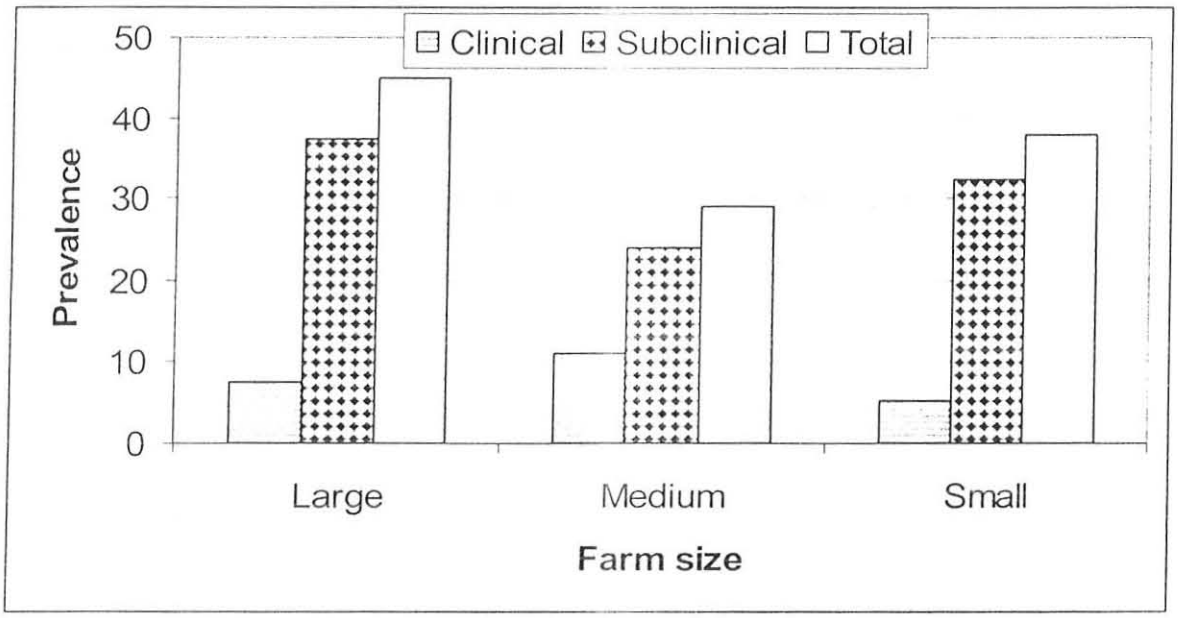


Figure 1. Clinical and subclinical mastitis on farm (herd) size

4.1.3. Risk Factors

Risk factors were investigated using binary logistic regression analysis in order to determine their association mastitis. The risk factors identified were farm size, parity, stage of lactation, hygiene, ventilation, drainage and quarter position. The association between the risk factors and occurrence of mastitis is presented in Table 7 and 8.

Table 7: Occurrence of mastitis related to risk factors

Risk factor	Total cows	Prevalence	P - value	χ^2	OR	95% CI for OR	
						Lower	Upper
Parity			.000***	33.55			
3	94	64.9			4.83	55.1	74.6
1	130	27.7				20.0	35.4
3	94	64.9			3.43	55.1	74.6
2	117	35.0				26.4	43.6
Stage of lactation			.006**	12.54			
1	82	54.9			2.52	44.1	65.7
4	155	34.2				26.7	41.7
1	82	54.9			2.72	44.1	65.7
3	55	30.9				18.7	43.1
1	82	54.9			1.37	44.1	65.7
2	49	46.9				32.9	60.9
2	49	46.9			1.98	32.9	60.9
3	55	30.9				18.7	43.1
Quarter position			.258	3.10			
Hind quarters	661	18.0			1.24	17.9	18.1
Front quarters	652	14.6				14.5	14.61

Table 8: Occurrence of mastitis related to risk factors

Risk factor	Total cows	Prevalence	P - value	χ^2	OR	95% CI for OR	
						Lower	Upper
Farm size			.103	3.40			
Large	225	44.9			2.12	37.5	50.5
Small	37	27.0				12.7	41.3
Large	225	44.9			1.35	37.5	50.5
Medium	79	36.7				26.1	47.3
Hygiene			.005**	9.96			
Poor	246	45.5			3.06	39.3	51.7
Good	28	21.4				6.2	33.6
Poor	246	45.5			1.96	39.3	51.7
Moderate	67	29.9				18.9	40.9
Ventilation			.001**	12.42			
Poor	248	46.0			3.69	39.8	52.2
Good	32	18.8				5.3	32.3
Poor	248	46.0			2.03	39.8	52.2
Moderate	61	29.5				18.1	40.9
Drainage			.020*	7.48			
Poor	264	44.3			2.65	38.3	50.3
Good	26	23.1				6.9	39.3
Poor	264	44.3			1.91	38.3	50.3
Moderate	51	29.4				16.9	41.9
Total	341	40.5				35.3	45.7

prevalence of mastitis, in parity 3 and 1 ($p<0.05$, OR=4.827). Thus, cows in parity 3 were 4.827 times exposed to mastitis than those cows in parity 1. Prevalence of mastitis in the first stage of lactation was statistically significant than in late lactation stage ($p<0.05$, OR=2.341). Cows in first lactation were 2.341 times exposed to mastitis than those cows in lactation 4. The difference between prevalence of mastitis in quarters with their anatomical position was not statistically significant ($p>0.05$, OR<1) however, prevalence of mastitis in hind quarters was higher than in front quarters (Table 9).

Table 9: Summary of binary logistic regression analysis of host risk factors

Risk factor	Group	SE	P value	OR	95% CI for OR	
					Lower	Upper
Parity			.000***			
	3	.292	.000***	4.827	2.725	8.550
	2	.276	.214	1.409	.821	2.418
	*1					
Stage of lactation			.006**			
	1	.279	.002**	2.341	1.354	4.045
	2	.333	.110	1.702	.887	3.267
	3	.337	.657	.861	.444	1.665
	*4					
Quarter position			.258			
	RH	.210	.079	1.445	.958	2.179
	RF	.202	.364	1.201	.808	1.788
	LH	.209	.099	1.410	.937	2.122
	*LF					

The prevalence of mastitis in the farms with poor hygienic condition was significantly higher than in the farms with good hygienic condition ($p<0.05$, OR=2.454). Prevalence of mastitis in farms with moderate hygienic condition was not significantly different with prevalence of mastitis in farms with good hygienic condition ($p>0.05$).

There was a statistically significant difference between the prevalence of mastitis in farms with poor and good ventilation ($P<0.05$, OR=3.687). Cows in farms with poor ventilation were 3.687 times exposed to mastitis than those cows in farms with good ventilation.

Logistic regression analysis indicated that statistically significant difference in the prevalence of mastitis ($p<0.05$, OR=2.653) in farms with poor drainage system than in farms with good drainage system. Cows in farms with poor drainage systems were 2.653 times exposed to mastitis than those cows in farms with good drainage system. Farm size was not significantly associated with mastitis ($p>0.05$), however the highest prevalence of mastitis (44.9%) on cow and (17.9%) on quarters was observed in large farms size (Table 8).

Table 10: Summary of binary logistic regression analysis of environmental risk factors

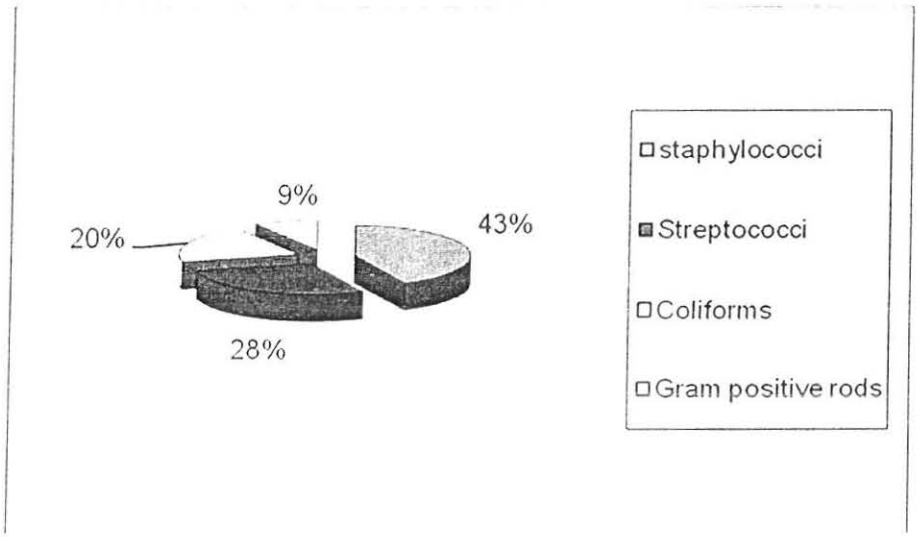
Risk factor	Group	SE	Sig	OR	95% CI for OR	
					Lower	Upper
Hygiene			.005			
	Poor	.478	.019	2.454	1.201	7.821
	Moderate	.532	.403	2.164	.550	4.429
	*Good					
Ventilation			.001			
	Poor	.470	.006	3.687	1.466	9.271
	Moderate	.533	.264	1.814	.638	5.155
	*Good					
Drainage			.020			
	Poor	.482	.043	2.653	1.032	6.820
	Moderate	.558	.556	1.389	.465	4.144
	*Good					
Farm size			.103			
	Large	.394	.056	2.121	.980	4.590
	Medium	.438	.305	1.566	.664	3.692
	*Small					

4.1.4. Etiological agents

During the present study 226 mastitis causing bacteria were isolated either singly or in combination from 214 positive milk samples. The most prevalent bacterial organisms isolated were *Staphylococcus* species (42.9%) following by *Streptococcus* species (28.3%) coliforms (20.0%) and other (8.8%). In both clinical and sub clinical cases *Staphylococcus aureus* and *Streptococcus agalactiae* were the highest number of isolates. Type, number and proportion of bacterial isolates were presented in Table 11.

Table 11: Mastitis causing microorganisms isolated from milk samples

Pathogens	Frequency	Percentage
Staphylococcus aureus	78	34.5
Streptococcus agalactiae	38	16.8
Escherchia coli	24	10.6
Streptococcus disgalactiae	13	5.8
Klebsiella	12	5.3
Corynebacterium pyogens	11	4.9
Staphylococcus intemedius	11	4.9
Streptococcus uberis	9	4.0
Corynebacterium bovis	6	2.6
Staphylococcus hycus	5	2.2
Proteus spp	5	2.2
Enterococcus faecalis	4	1.8
Enterobacter spp	4	1.8
yersinia	3	1.3
Bacillus cereus	3	1.3
Total	226	100



Fiure 2. Proportion of isolates on genera level

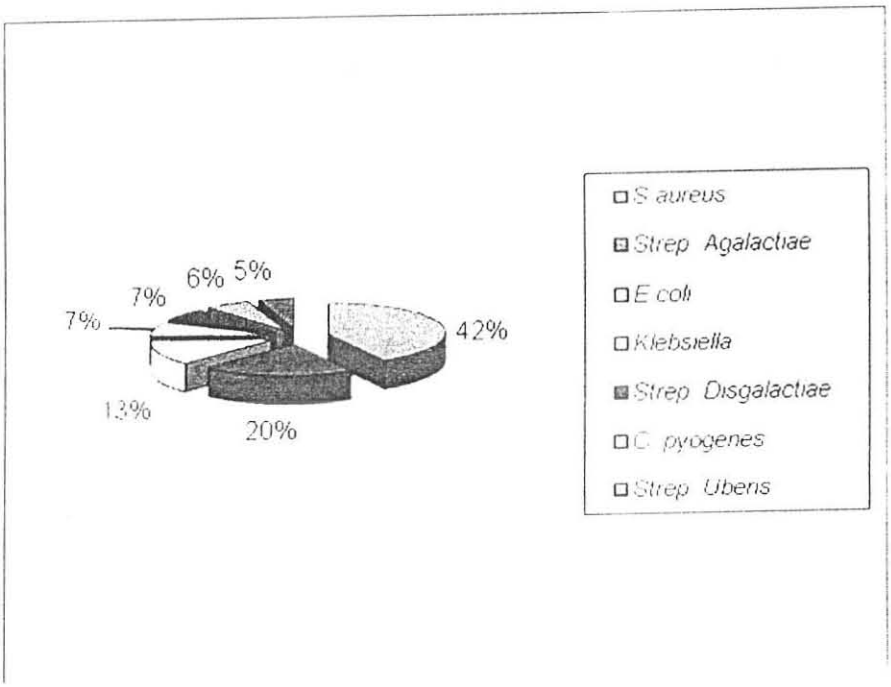


Figure 3. Proportion of major Pathogens

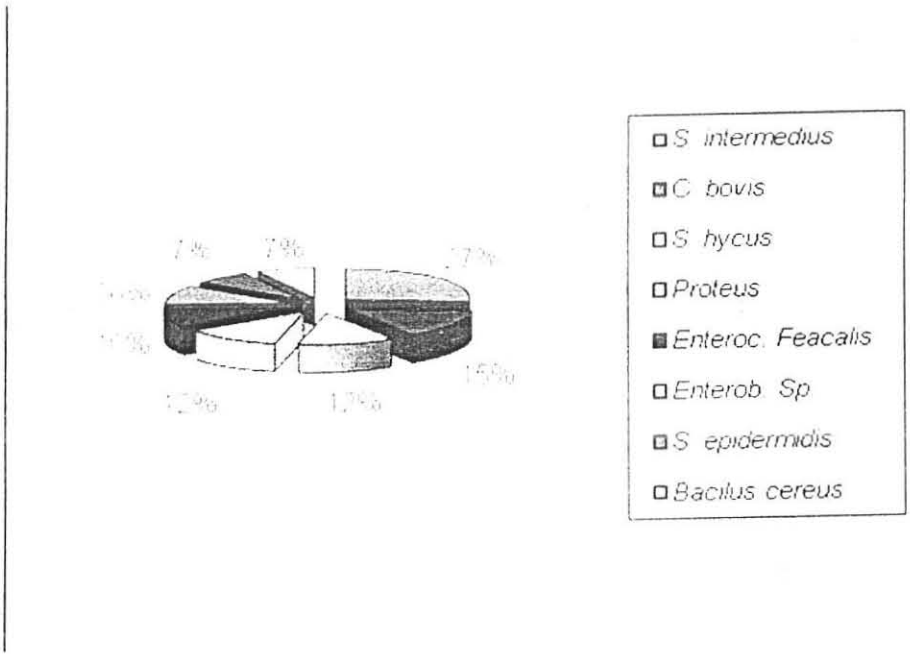


Figure 4. Proportion of minor pathogens

4.1.5. Invitro Antimicrobial Sensitivity Tests

A total of 121 isolates from clinical and sub clinical mastitis were tested for their in vitro antibiotic susceptibility against seven antimicrobial drugs. The drugs were commonly used to treat farm animals for mastitis and other infectious diseases.

Drug sensitivity test showed that the majority of isolates were highly sensitive to cloxacillin, gentamycin and amoxicillin. *Corynebacterium* group *klebsiella* and *proteus* were moderately sensitive to ampicillin and oxytetracycline. However, *Staphylococcus* and *Streptococcus* species were highly resistance to streptomycin and penicillin (Table 12).

Table 12: Antibiotic sensitivity of etiological agents isolated from cases of mastitis

Pathogens	Total isolates	No. of tested	OXY	GEN	STR	AMP	AMO	PEN	CLO
S. aureu	78	40	22/18	36/4	10/30	21/19	35/5	9/31	38/2
Strep. agalactiae	38	20	11/9	17/3	8/12	10/10	18/2	5/15	19/1
Strep. disgalactiae	13	10	4/6	8/2	4/6	4/6	7/3	2/8	9/1
S. intermedius	11	6	2/4	5/1	3/3	4/2	5/1	3/3	5/1
Strep. uberis	9	6	2/3	5/0	2/4	3/3	4/2	2/4	4/2
E. coli	24	15	8/7	12/3	10/5	8/7	12/3	6/9	13/2
Klebsiella	12	8	5/3	5/2	4/4	6/2	5/3	4/4	6/2
C. Pyogens	11	7	5/2	6/1	3/4	4/3	5/2	4/3	6/1
C. bovis	6	4	2/2	3/1	1/3	3/1	3/1	2/2	4/0
Proteus	5	5	3/2	3/2	3/2	4/1	4/1	2/3	4/1
Total	207	121	65/56	101/20	48/73	67/54	98/23	39/82	108/13

OXY - Oxytetracycline (30 µg) STR - Streptomycin (10 µg) PEN - Penicillin (10 IU)

GEN - Gentamycin (5 µg) AMP - Ampicillin (2 µg) CLO - Cloxacillin (5 µg)

AMO - Amoxicillin (10 µg) S/R - Sensitive/Resistant

4.2. Questionnaire Survey

4.2.1. Farm distribution

The study was conducted in thirty-six dairy farms found in four Districts and seven Kebeles. Most of small size farms were located in peri-urban areas. The majority of the medium and all large size farms except one were located in urban areas (Table 2).

4.2.2. Farm structure and social aspects

The occupational status of the owners range from business men/women to house wife and retired people. Only eight (22.2%) of them were fully engaged in the dairy activity. The educational level of the farm owners also vary from degree holders to some grade school. Two (5.6%) farm owners were holder of degree, five (13.9%) holder of diploma and the rest 29 (80.5%) had an educational level of high school and some alternated elementary school. However, farms operated by degree, and diploma holders were equally affected by mastitis as those of others having lesser level of school education. Among the farm owners 11 (30.6%) were females. The majority 27 (75%) of the farms were shared with the living compound of owners.

The availability of the dairy workers was adequate in most of the farms, however, lack of knowledge and skill of employed workers was complained by the majority (66.6%) of the farm owners. The most common sources of information about the dairy farms were animal health personnels, animal production professionals or extension agents. Nine (25%) of the farm owners had an extra land for the production of animal feed but all dairy farm owners indicated the feed shortage due to reduced supply and increased cost. All farms utilize potable water, which was clean and supplied adlibitum (Annex 8. 3).

4.2.3. Health problems and management measures

The most frequent health problems of all dairy farms were reproduction failure and mastitis in order of importance. All cows in all farms were milked twice a day (morning and evening). Cows

are milked by stripping. Milking machine is not used in all of the farms. The most common problems for culling of cows were reproductive failure (36.1%), mastitis (27.8%), aging (19.4%) and reduced production (16.7%). High cost of feed was also mentioned as the other reason for culling in order to reduce the number of herd size.

Almost all small farm holders used family labor for milking and farm management. Medium and large farm owners used paid labor with a small share of family labor. In all farms udder and hands of milker are washed prior to milking. However, disinfectants are not used in all the dairy farms and soap is not regular. Except in one farm all milkers in all 35 farms used one common towel or rag for several cows to dry the washed udders and teats. Other mastitis prevention and control measures like post milking dipping, dry cow therapy, washing of milkers hands between milking were not used in all dairy farms. In 17 (47.2%) farms cows with mastitis are milked at any time (rather than milking after healthy ones). Twenty two (61.1%) farm owners responded that mastitic cows are not treated on time (Table 13).

Table 13: Result of questionnaire survey on milking cow management, mastitis prevention and control measures

Questions	Respondents	Percentage
Knowledge and skill of dairy workers		
Satisfactory	2	5.5
Moderate	10	27.8
Unsatisfactory	24	66.7
Udder and washing before milking		
Yes	36	100
No	0	0
Udder drying		
Yes	36	100
No	0	
Use of separate towels		
Yes	1	2.8
No	35	97.2
Teats dipping		
Yes	0	0
No	36	100
Time of milking cows with mastitis		
Last	19	52.8
Any time	17	47.2
On time treatment of mastitic cow		
Yes	14	38.9
No	22	61.1
Practicing dry cow therapy		
Yes	0	0
No	36	100
Knowledge of dry cow therapy		
Yes	0	0
No	36	100

Record keeping for individual animals was not practiced in all farms, but nine farms had a record for milk production on farm level (Annex 8.3). Therefore, whenever information about the farms was needed either the owner or the dairy workers are consulted. Whenever their cows get mastitis, they consult animal health personnel. The common drugs available in veterinary clinics and in the market are used haphazardly. The drugs are penicillin, streptomycin, oxytetracycline, gentamycin, cloxacilli ampicillin and amoxicillin (Annex 8.4). All farm owners complained about the high price of drugs. All farmers also complained that drugs fail to cure mastitis.

4.2.4. Farm inspection, management and host factors

All 100% housing type are tie stall, 28 (77.8%) are modern (made of cement, iron sheet and timber perlins) and the rest are mixture of modern and traditional (made of wood and mud). Floor type is concrete in 32 farms (88.9%) while the rest 3 (11.1%) are stone. Farms with earthen floor are not observed in this study. Stall space is inadequate in 19.1% of houses and doors in all farms are free of defect. Roofs are rainproof; however, some houses have an opening at list on one or more sides of the house under the roof. Therefore, in such conditions, rain can enter the house exposing animals to cold weather and wetting the floor..

Seventeen (47.2%) dairy farms had poor, 10 (27.8%) moderate and 9 (25%) good hygienic condition. Infrequent cleaning of animal house and improper disposal of waste material were the major problems seen in almost all farms of poor hygiene. Statistical analysis indicated that poor hygiene was significantly associated with mastitis ($p=0.005$). Ventilation was also highly related to mastitis prevalence ($p=0.001$). Eighteen (50%) of farms investigated had poor, 10 (27.8%) moderate and 8 (22.2%) good ventilation. Some of the houses had small size windows, which were not proportional to the houses area, and this can result in humid and hot condition inside. Twenty (55.6%) of the farms had poor, 8 (22.2%) moderate, and 8 (22.2%) good drainage system. Accumulation of waste materials was observed outside the animal house near the gates and in the exercise yard. Drainage was also significantly associated with the occurrence of mastitis ($p=0.020$). There were cows observed with serious and apparent soiling of udder, which was related to poor hygienic, and drainage standard of the farms (Annex 8.4).

5. DISCUSSION

The overall prevalence of mastitis obtained in this study was 40.5% and this result agrees with findings of kerro and Tareke. (2003) who reported 40% prevalence in southern Ethiopia, and Abdella *et al.* (1996) who obtained a prevalence of 39% in Wondogenet. Prevalence of clinical and subclinical mastitis for this study was 6.8% and 33.7%, respectively. Relatively similar result was reported by Bishi (1998) who recorded 5.5% clinical and 30.2% sub clinical and by Mungube *et al.*(2004) also observed 6.6% for clinical mastitis in Addis Ababa. The 16.3% quarter infection rate investigated in this study was also similar to the result of Abdela (1996) which was 16% but lower than the 19% quarter infection reported by Kerro and Tareke (2003) and 25% indicated by Radostits *et al.* (2000).

Quarter infection for clinical and sub clinical mastitis for this study was 3.2% and 13.2% respectively, the prevalence of clinical mastitis on quarter level observed in this study was almost similar to that of Mungube *et al.* (2004) who reported 2.8% for clinical mastitis on quarter level.

Prevalence of mastitis in single quarter affected cases was higher than multiple (double and triple) quarter infection. In line with this Thennarasu *et al.* (2003) observed that incidence if mastitis in single quarter affected cases was highly significant in cows and buffaloes than multiple quarter infection

Hind quarter infection was higher than front quarter even though statistically insignificant. This would be due to frequent exposure of hind quarters to effluent than the front quarters. This is in agreement with the finding of Thernnarasu (2003) who reported that incidence of mastitis in cows and buffaloes was higher in hind quarters than the front quarters. How ever, there was no visible difference between the left and the right quarters.

The analysis of risk factors for mastitis indicated that parity, stage of lactation, hygiene, ventilation and drainage were significantly related to mastitis occurrence. Similar observation has been reported by Mungube *et al.* (2004) and Kivaria *et al.* (2004). In this study, significant difference in mastitis prevalence was observed between different herd sizes. The highest mastitis

prevalence was established for the large farms with large herd size. This is in line with Bishi (1998) and Radostits (2001) who indicated that as herd size increases manure disposal and sanitation problem was also increases but contrary Nesru (1999) and Mungube (2005) reported that higher prevalence in small-scale farms. Frese (1999) found that large farms located in urban areas with limited operational space are associated with hygienic risk factors that may have significant contribution to the emergence of mastitis problem. Small holder dairy farms are generally more efficient than large scale farms in most urban and peri-urban areas (Shem *et al.*, 2001). In addition, in this study it was observed that the use of family labor in small dairy farms may contribute by providing better attention to farms.

This investigation showed that prevalence of mastitis was lower during the first parity and higher in the late parity. Young animals have a decreased susceptibility through a more effective host defense mechanisms while older cows, are more prone to mastitis. The increased prevalence of mastitis with increasing parity number is in agreement with findings by Sargent (1998); Bustan *et al.* (2000) and Kerro and Tareke (2003) found that the risk of clinical and sub clinical mastitis increased significantly with advanced age of the cow, which approximates with parity number.

The frequency of mastitis was higher during early lactation as compared to late lactation stage. In line with this result, Nesru (1999) and Kerro and Tareke (2003) reported higher prevalence of mastitis during early lactation than late lactation. This is in agreement with the report of Thennarasu (2003) who found that effect of lactation stage on the occurrence of mastitis in cows increased during the first and the second lactation period. The occurrence of more cases during the first lactation stage may be due to absence of dry period therapy and birth related influences (Quinn *et al.*, 2005). In this study, the prevalence of mastitis was slightly higher during the fourth lactation stage than the third. Similarly Kerro and Tareke (2003) and Rodostits *et al.* (2000) suggested that the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which may in turn increase the number of potential pathogens on the skin of the teat. In line with this Quinn *et al.* (2005) asserted that many infections caused by environmental pathogens occur during the dry off period and on the weak before calving.

Of the farms studied, three-fourths had poor and moderate hygienic condition. In general unhygienic milking procedures, un improved management, wet stalls due to infrequent cleaning, the absence of post milking teat dip, lack of proper treatment for clinically infected cows and absence of dry cow therapy can be predisposing factors for increased infection rates in all group of cows. This finding hence, agrees with observations made by Shem (2001), Barkema *et al.* (1999) and Redeat (2009) who indicated about the significant role of poor farm management and unhygienic milking procedures on the occurrence of mastitis.

Udder and milkers hands were washed before milking in all farms however, most of the time milkers used only water to wash hands and udder, soap was not always considered as a compulsory. Disinfectants were not used in all surveyed farms. Use of water only may reduce pathogenic microorganisms but does not guarantee their elimination. Over all poor milking hygiene may result in increased exposure and transmission of mastitis pathogens during milking (Kivaria *et al.*, 2004). In almost all of the study farms milkers used single towel for udder preparation. This can facilitate the transmission of pathogens from cow to cow and increases the frequency of mastitis occurrence. Similarly Dargent-Molina *et al.* (1998) reported that the protective effect against mastitis of using separate towel for drying teats is a well-established method in reducing spread of contagious pathogens.

From the total farm owner half were business men/women and civil employee, their dairy farms were sideline jobs, only to add to their income from other major occupation, only twenty-two percent of them were full time dairy farmers. This was in view of exerting full time labor and endeavoring to draw maximum benefit out of their effort (Nesru, 1999). Thirty percent of the dairy owners were females. This may show the increasing trend in Ethiopia that they are attached to dairy activities like in most areas.

The majority (80.6%) of the farm owners has attended from elementary school to collage level however; the educational standard did not seem to have an observed effect on dairying in the farms studied. Even those academically well-trained farm owners did not take advantage of their skill and professional to improve their farm management. Their farms were equally affected as those belonging to owners with very low educational level. This fact indicated the need for a

consistent extension services to address farmers on dairy activities. 66.6% of the dairy farm owners were complained about the unsatisfactory skill and knowledge of dairy workers while 27.2% of the owners were moderately satisfied.

Even though, the availability of dairy workers in the majority of farms was adequate the low skill and knowledge of the workers about the dairying and the negligence of the owners may have negative impact on the management and hygiene of the farms. Dairy workers who were milking cows were involved in other farm activities such as barn cleaning, feed distribution and managing of other group of animals. Lack of knowledge about dairying among dairy operators and their involvement in other farm duties had resulted in poor hygienic and inadequate udder preparation, consequently leading to mastitis (Kivaria, 2004). Therefore, this may favor the dissemination of environmental mastitis causing microorganisms and increasing the frequency of environmental mastitis.

Almost all small dairy holders use family labor for handling and milking of their animals. In agreement with this Nesru (1999) reported that involvement of family members in dairy activities may contribute to give better attention for the good handling of their farms. In the medium and large size farms used paid labor as well. Farms obtained information from veterinarians, dairy professionals or extension agents. Radostits *et al,* (2001) stated that farm record is considered as an essential tool to manage disease, breed, feed, production and culling of animals. However, none of the studied farms had such type of record, except 9 farms which were recorded milk production on farm level. The two most frequent health problems mentioned in all farms were reproduction failures and mastitis. Due to failure of repeated insemination, about one-tenth of cows were milked more than a year. This may result in economic loss due to the delay in calving. High cost of feed was also mentioned as the reason for culling in order to reduce the number of herd size. Five small and two medium farm owners located in the peri-urban areas were owned extra land. They grow feed and provide crop residues, however, like other farms fulfilled extra need from the market. The majority of farms did not considering mastitis as the reason for culling of cows which poses serious risk of infection for clean cows and also poses economic loss to the farmers. Culling of cows with chronic mastitis is the most effective method of decreasing new intra mammary infection (Quinn *et al.,* 2005).

In all dairy farms housing system is tie stall, only about half of the farms gave exercise to their animals. The rest kept their animals in total confinement where cows are restricted to the small areas that were dirty and wet. The exposure of teat to dirty and wet environment with excessive moisture, probably resulted in the increased intra mammary infection (Kivaria *et al.*, 2004). Those cows found in farms exercised their animals may get sunlight during exercise time, but the rest did not have access to sunlight and failed to make use of it. It is worth mentioning that this condition can favor excess moisture and allow the growth of environmental organisms. About half of the farms milked their mastitis cows at any time during the milking hours rather than milking after the healthy ones. Milking of cows with mastitis at any time during milking hours obviously facilitates cow-to-cow transmission of contagious as well as environmental pathogens (Matos *et al.*, 1991).

The farms with concrete floor allow adequate cleaning and not hazars. In contrast floor with arranged stone and crack is clearly a risk and not suitable for cleaning. Almost all farms had rainproof roofs, however, those houses with a wide and long opening under the roof might allow rain to the house wetting the stalls.

The study revealed that *Staphylococcus aureus* was the most important pathogen associated with bovine mastitis. This agree with findings of Radostits *et al.* (2000) who asserted that *Staphylococcus* is well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it is shed in milk facilitating transmission to healthy animals mainly during milking procedures.

Agents of environmental pathogens or "coliforms" were isolated on the third level with contagious pathogens. Therefore, detection of both groups of pathogens is an indicator for the presence of un hygienic farm and milking management in the study dairy farms. The measures appropriate for the prevention and control of bovine mastitis differ depending on weather the causative organisms are contagious or environmental in origin (Quinn et al., 2005) The result on the rates of isolates obtained in this study also agrees with the findings of various authors (Mulei, 1999; Wage *et al.*, 1999; Workineh *et al.*, 2002; Kerro and Tareke 2003; Nesru, 1999 and Bishi,

1998) who reported *Staphylococcus, Streptococcus* and coliform species were the predominant isolates of clinical and subclinical mastitis pathogens.

It was observed that many owners of dairy farms used drugs for treatment without rational prescription. The invitro disk sensitivity test provided a result that can be guidance for the studied dairy farms in order to choose an effective antibiotic to treat mastitis. Drug sensitivity test showed that microbial isolates wee highly sensitive to cloxacillin, gentamycine and amoxicillin and moderately sensitive to ampicillin and oxytertracycline. Whereas Staphylococcus and streptococcus species showed the wide spectrum resistance to streptomycin and penicillin which may be due to the excessive and prolonged use of these antibiotics for mastitis and other infectious diseases in the farms. The effectiveness of cloxacillin detected in this study agrees with the findings of Quinn *et al.* (2005) who asserted that cloxacilli is an effective drug against *Staphylococcus aureus, Streptococcus agalactiae, Escherchia coli* and environmental *Streptococci.*

6. CONCLUSION AND RECOMMENDATION

The high prevalence of mastitis obtained in this study indicated that bovine mastitis is one of the major disease problems in dairy farms in the study area. The frequency of subclinical mastitis was higher than clinical mastitis. Dairy owners and workers were not aware of subclinical type of mastitis. Efforts have been concentrated on the treatment of clinical cases while the high economic loss could come from sub clinical mastitis. In most of dairy farms studied, the hygienic conditions were poor. The standard of milking procedure was also very low. Generally mastitis prevention and control measures were not properly practiced in the majority of the dairy farms. Confinement of animals in poorly ventilated houses and with out giving exercise was commonly observed. Low level of knowledge about dairy management was seen on the dairy workers and visible negligence of owners has also a contribution for the observed farm management problems. *Staphylococcus aureus* was the predominant organism isolated from both clinical and subclinical cases followed by *Streptococcus agalactiae* and *Escherchia coli.* The isolation of contagious and environmental mastitis causing bacteria in combination is indicator for the presence of poor farm and milking hygienic condition.

According to the drug sensitivity result cloxacillin, gentamycin and amoxicillin are the most effective drugs to treat mastitis in the study dairy farms. However, *Staphylococcus* and *Streptococcus* species which are considered as major pathogens were highly resistance to streptomycine and pencillin therefore, use of these drugs is not recommendable.

Based on the above concluding remarks the following recommendations are forwarded:

- There should be an implementation of mastitis prevention and control strategies including record keeping and provide advice and training regularly on basic farm and milking cow management, economic and public health importance to the owners and dairy operators.

- Creation of awareness about the presence of invisible (sub clinical) type of mastitis and techniques of it's screening.

- Antibiotic susceptibility test prior to the use of antimicrobial drugs should be practiced in both treatment and prevention of intra mammary infections.

- Longitudinal study followed by interventional studies is also recommended in order to determine the importance and role of risk factors in the occurrence of mastitis.

7. REFFERENCE

Abdella, M. (1996): Bacterial causes of bovine mastitis in Wondogenet. *J. Vet. Med.*, **43** (6):379-384.

Bansan, B. K., Joernhaman, N. T., Crabwoskit and Krishan, B. S. (2005): Variation in Composition of Selected Milk Fraction Sample from Healthy and Mastitic Quarters. *Journal of Dairy Reaserch*, **72**:144-152.

Barkema, H. W., Schukken, Y. H., Lam, T. J., Beiboer, M. L., Bened, Ctus, G. and Brand, A. (1999): Management practices associated with the incidence rate of clinical mastitis. *Journal of dairy Science*, **82**:1643-1654.

Bishi, A. B. (1998): Cross sectional and longitudinal prospective study of bovine clinical and sub-clinical mastitis in peri-urban and uban dairy production system in Addis Ababa Region, Ethiopia. MSc Thesis, Freie University Berlin and Addis Ababa University, Berlin, Germany.

Bradley, A. J. (2002): Bovine Mastitis, an Evolving Disease. *The Veterinary Journal,* **164**:116-128.

Bustan, A., Trachsel, P., Schallibaum, M. and Blun, J. M. (200): Udder health and risk factors for subclinical mastisti in organic dairy farms in Swizerland. *Preventive Veterinary Medicine,* 44:205-220.

Central Statistics Authority (CSA) (2008): Statistical Abstracts 2007. Addis Ababa, Ethiopia.

Coles, E. H. (1986): Veterinary clinical pathology. 4th Edition, W.B. Saunders Company, Philadelphia, Pp 360-369.

Cullor, J. S. (1991): The role of vaccines in the prevention and moderation of clinical mastitis. Proceedings of the thirteenth annual meeting of the National Mastitis Control, Reno, Nev. USA.

Dargent-Molna, P., Searlett, J., Pollock, R. V. H., Erb, H. N. and Sears P. (1998): Herd level risk factors for *Staphylococcus aureus* and *Streptococcus agalactiae* intramammary infections, *Preventive Veterinary Medicine*, 6:127-142.

Deluyker, H. A. (2005): Factors Affecting Cure and Somatic Cell Count After Pirilimycine Treatment of Sub clinical Mastitis in Lactating Cows, *Journal of Dairy Science,* **88**:604-616.

Demelash, B., Etana, D. and Fekadu, B. (2005): Prevalence and risk factors of mastitis in Lactating dairy cows in southern Ethiopia. *J. APPL. Res. Vet. Med.*, **3:**200.

Dulin, A. M., Paap, M. J. and Nickerson, S. C. (1988): Comparison of phagocytosis and chemiluminesescence by blood and mammary gland neutrophils from multiparous and mulliparous cows. *J.Am. Vet. Res., 49:172-177.*

Dwight, C. H. and Yuan, C. Z. (2002): Veterinary Microbiology. Black well science Tokyo, Pp 251-257.

Edward, M., Anna, K., Michael, K. Z., Henry, K. and Krystina, S. (2002): Antimicrobial Susceptibility of Staphylococci Isolated from Affected with Mastitic Cows. *journal veterinary natural spillway,* **46**:189-294.

Ellen, J. B., Lancer, P. and Finegold, M. (1994): Diagnostic Microbiology. Toronto, Mosby, Pp 701-703.

Erskine, R. J. (2001): Mastitis control in dairy farms in Herd Health, In: Food animal production Medicine, 3rd Edition, W. B. Sunders Company, Pp 397-435.

Frese, M. (1999): Cross-site and cross-location on farm investigation on epidemiology of mastitis in market oriented urban/peri-urban production system in the region of Addis Ababa and Debrezeit, Ethiopia. Diploma Thesis, Freie University of Berlin, Germany.

Food and Agricultural Organization (FAO), (2003): Milking, milk production hygiene and udder health. Animal production and health paper, series 78. FAO of United Nations, Rome, Italy, Pp 68-72.

Getahun, K. (2006): Bovine mastitis and antibiotics resistance patterns of major pathogen in small holder dairy farms in central high lands of Ethiopia. MSc Thesis, Addis Ababa University, Debrezeit, Ethiopia.

Gonzalez, R. N., Cullor, J. S. and Jasper, D. E. (1989): Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherchia Coli* vaccine. *Can J. Vet. Res.*, **53**:301-305.

Gruent, P. (2001): Bovine Mastitis and Intramammary Drug Delivery Review and Perspectives, Advanced Drug Delivery Reviews, **50**:245-259.

Harari Regional Agricultural and Rural Development Bureau (HRARDB): Annual report on animal health and production activities. Harar.

Kerro Dego and Tareke, F. (2003): Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health and production*, 35(3):197-205.

Kivaria, F. M., Noordhuizen, T. M. and Kapanga, A. M. (2004): Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Tropical Animal Health and Production*, **36(6):**581-591.

Lema, M., Kassa, T. and Tegene, A. (2001): Clinically manifested major health problems of cross bred daliry herds in urban and peri-urban production systems in the central highlands of Ethiopia. *Tropical Animal Health and Production*, **33**:85-93.

Marion, B. R. (2007): Plant Produced Proteinases Dairy Cow Infection, High Beam Encyclopedia Research. *American Veterinary Medical Association.* Pp 12-53.

Marthengo, J. M. and Ombui, J. N. (2007): Detection of staphylococcus entrotoxins in milk and meat in Nirobi Kenya. *Journal Tropical Microbiology,* **1(3):**23-38.

Matos, J. S., White, D. G., Langlois, B. E. (1991): Isolation of *Staphylococcus aureus* From sites other than the lactating mammary gland. *Journal Dairy sci.* 74:1544.

Mifflin, M. (2004): Bovine Mastitis-Definition of Bovine Mastitis in Medical Dictionary, the Free Dictionary by FARLEX. Pp 15-20.

Minna, K. (2004): Genetic and Phenotypic Relationships among Milk Yield and Somatic Cell Count Before and After Clinical Mastitis, *Journal Dairy Science,* **88**:827-833.

Morison, W. I., Murray, M. and Mentyre, W. I. (1991): Disease of cattle in the tropics. Current Veterinary Medicine and Animal Science. Farming Press Limited, London, Pp 469-480.

Mulei, C. M. (1999): Teat lesions and relationship to intramammary infection on small scale dairy farm in Kiambu district in Kenya. *Journal of the south Africa veterinary Association,* **70**:156-157.

Mungube, E. O., Tenhagen, B. A., Regassa, F., Kuyle, M. N., Sheferaw, Y., Kassa, T. and Baumann, M. P. O. (2005): Reduced milk production in udder quartes with subclinical mastitis and associated economic losses in cross bred dairy cows in Ethiopia. *Tropical Animal Health and Production,* **37(6)**:503-512.

Mungube, E. O., Tenhagen, B. B., Kassa, T., Regassa, F., Kuyle, M. N., Geriner, M. and Baumann, M. P. O. (2004): Risk factors for dairy cows mastitis in the central highlands of Ethiopia. *Tropical animal Health and production*, **36(5)**:463 472.

Mungube, E. (2001): Management and Economics of Dairy Cow Mastitis in Urban and Peri-urban areas of Addis Ababa Milk Shed, MSc Thesis, Addis Ababa Univeersity, Faculty of Veterinary Medicine, Debre Zeit , Ethiopia.

Nibret, (2007): Prevalence of bovine mastitis in lactating cows among small dairy herds and antimicrobil sensitivity test on major isolated pathogens in and around Gonder. MSc Thesis, Addis Ababa Univesity, Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Nelson, W. P. and Stephan, C. N. (1991): Mastitis counter attacks. Babson. Bros. Co.Naperville, Pp18-59.

Nesru, H. (1999): Cross sectional and longitudnal study of Bovine mastitis in urban and peri-urban dairy systems in the Addis ababa region. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine Debrezeit, Ethiopia.

Phillips, C. J. (1996): Progress in dairy science. Farming Press Limited London, Pp 177-195.

Phuekets, P., Monsell, P. D. and Browning, G. F. (2002): Multiplex PCR assay for simultaneous detection of *Staphylococcus aureus* and streptococcal cases of bovine mastitis. Cornell University Press, New York, Pp 27-29.

Quinn, P. J., Markey, B.K., Donnelly, W. J., Leonard, F.C. and Maghire, D. (2005): Veterinary microbiology and microbial disease. Blackwell science Ltd. UK, Pp 465-475.

Quinn, P. J., Leonard, F.C., Maghire, D., Markey, B.K., Carter, W. E. and Donnelly, W. J. (2002): Clinical veterinary microbiology. Wolfe, Baltimore, Pp 327-344.

Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (1999): Mastitis in Clinical Quinn, P. J., Carter, G. R. and Markey, B. (1994): Mastitis. In. Quinn, P. J. (ed.), Clinical veterinary microbiology. Wolfe, Baltimore, Pp 327-334.

Quinn, P. J., Carter, G. R. and Markey, B. (1994): Clinical veterinary microbiology. Wolfe, Baltmore, Pp 327-344.

Radostits, O. M. (2001): Herd Health, Food Animal Production Medicine. 3rd Edition, W. B. Sounders Company, Philadelphia, Pp 397-428.

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

Radostits, O. M., Leslie K. E. and Fetraw, J. (1996): Mastitis control in dairy herds. In: Radostits, O. M. Edition, Herd Health, Food animal production medicine. 2nd Edition, W. B. Aunders Company, Philadelphia, Pp 229-276.

Redeat, B. (2009): Microbiological study on bacterial causes of bovine mastitis and it's antibiotic susceptability patterns in east Shoea zone Oromia regional state. MSc Thesis, Addis Ababa University, faculuty of Veterinary Medicine, Debrezeit, Ethoipia.

Sargent, J. M., Scatt, H. M., Lestie, K. E., Irland, M. J. and Bashir, A. 1998): Clinical mastitis in dairy cattle in Ontario, frequency of occurrence and bacteriological isolates. *Canadian veterinary Journal*, **34(33)**:240.

Savasan, S. (2002): clinical examination a rapid detection of gram positive and gram negative bacteria in bovine mastitis. Year book publishers, Inc. London, Pp 23-27.

Schroeder, J. W. (1997): Mastitis Control Program. Bovine Mastitis and Milking Management, North Dakote State University (NDSU) Press. Pp 17-49.

Shem, M. N., Malole, J. M.L., Machangu, R., Krwijila, L. R. and fujihara, T. (2001): Incidence and causes of subclinical mastitis in dairy cows on smallholder and large scale farms in tropical areas of Tanzania. *Asian Australian Journal of animal science,* 14(3):372-377.

Suriyasathaporn, W., Schukan, Y. H. N., Nilson, M. and Brand, A. (2000): Low somatic Cell count, a risk factor for subsequent clinical mastitis in a dairy herds. *Journal of dairy Science,* **83**:1248-1255.

Tamrat, T. (2006): Comparison of clinical trial of bovine mastitis with the use of antibiotics and honey in Sodo area, Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debrezeit, Ethiopia.

Thennarasu, A., Muralidharium, M. R. and Murugan, M. (2004): Effect of lactating parameters on the incidence of bovine mastitis. India Journal of animal science, 74(4):379-381.

Thrusfield, M. (2005): Veterinary epidemiology. 2nd Edition, Blackwell Science Limited, United Kingdom, Pp 178-183, 225-227.

Wage, S., Mork, T., Roros, A., Asland, D., Hunshamar, A. and Odeggard, S. A. (1999): Bacteria associated with subclinical mastitis in dairy heifers. *Jurnal of Dairy science.*, **82**:712-719.

Wellenberg, G. J., Vanderpoel H. M and Vanoirschot, J. T. (2002): Viral infection and bovine mastitis, *Journal of Veterinary Microbiology,* **88**:27-45.

Workineh, S., Bayleyeng, M., Mekonnin, H. and Potgieter, L. N. D. (2002): Prevalence and etiology of mastitis in cows from two major Ethiopian dairies. *Trop. Anim. Helth. Prod.,* **34**:19-25

8. ANNEX

8.1. Questionnaire

A. Herd description and Farm structure

Farm No _____

Farm owner _____ Sex _____

Farm address: Town _____ Kebele _____ Tel _____

Urban _____ Pre-urban _____

Farm size _____

Total number of cattle in the farm _____

Total number of each group: Cows _____ Heifers _____

Bulls _____ Oxen _____

Calves _____

Breeds of cattle owned _____

B. General production information

Number of dairy cows in a herd on present: _____

Number of cows of each breed that are milking on present:

Local zebu _____Cross breed _____ Pure exotic_____ Total _____

Number of milking cows according to stage of lactation:

First lactation _____

Second lactation _____

Third lactation _____

Fourth lactation _____

Fifth lactation _____

Six and above lactation _____

Number of milking cows according to parity

Parity one _____

Parity two _____

Parity three_____

C. Health problem

What are the most frequent diseases encountered in the farm?

On descending order:

1 _____ 3 _____ 5 _____

2 _____4 _____ 6 _____

What are the reasons for culling of cows?

_____ _____ _____

D. Social structure

Owner's occupation _____

Education level _____

Number of dairy workers _____

Occupational capacity and skills of dairy workers

Satisfactory _____ moderate _____ unsatisfactory _____

Availability of dairy workers

satisfactory _____ moderate _____ unsatisfactory _____

Are there owners family members participating on farm activities? Yes _____ No _____

Do you have farm record for? Yes _____ No _____

Do you give exercise to the animals? Yes _____ No _____

What are sources of dairy farm information?

_____ _____ _____

_____ _____ _____

E. Milking procedure and management

How many times cows are milked in a day? _____

Are teats and udder cleaned and washed before milking? Yes_____ No _____

Are udder and teats dried before milking? Yes _____ No _____

Are teats dipped after milking? Yes _____No_____

Is antiseptic used for dipping? Yes _____No _____

Is a teat dip used for each cow? Yes _____ No _____

Are hands of milkers washed before milking? Yes _____No _____

Are separate towels used for each cow? Yes _____No _____

Is a disinfectant used for washing hands? Yes _____No _____

Is a dry cow therapy practiced? Yes _____ No _____

Are mastitis cases (clinical) treated on time? Yes _____ No _____

Is there any screening test used? Yes _____No _____

When do you milk cows with mastitis? _____

F. Farm inspection report

Housing system

Pen _____ Station _____ Barn _____ Free stall _____

Floor

earth _____ concrete _____ stone _____

Wall

concrete _____ mud _____ Metal sheet _____

Roof

metal sheet _____ grass _____ other _____

Drainage system

good _____ moderate _____ poor _____

Ventilation

good _____ moderate _____ poor _____

Doors and windows

good _____ moderate _____ poor _____

General farm hygiene

good _____ moderate _____ poor _____

Cow cleanliness

good _____ moderate _____ poor _____

Are cow stalls of adequate size? Yes _____ No _____

Are cow stalls free of hazards? Yes _____ No _____

Is the roof structure rainproof? Yes _____ No _____

G. Feed and water

Do you have an extra land for animal feed production?

Yes _____ No_____

Are feed stuffs available for the animals?

Yes _____ No _____

Is water available for the animals?

Yes _____ No_____

----------/ /----------

----------/ /----------

8.2. Sample collection format

Region _____ Town _____ Kebele _____ Farm No _____

Type of sample _____

Date of sample collection _____

Date of test _____

Purpose _____

Sample collection and CMT result registering format

Farm (Herd) No	Cow No	Sample ref. No	Breed	Age	Calving	Stage of lactation	Quarter				Remark
							RF	RH	LF	LH	

8.3. Summary of questionnaire survey on social aspects and animal management

Detail	Urban			Peri-Urban			
	Small	Medium	Large	Small	Medium	Large	Total
Sex of owners							
Male	5	4	6	4	4	2	25
Female	5	4	1	1	-	-	11
Occupation							
Full time dairy farmer	3	1	-	2	-	2	8
Business men/women	7	2	3	-	1	-	13
House wife	-	4	-	-	-	-	4
Employee	-	-	2	2	-	-	4
Regular (mixed) former	-	-	-	1	3	-	4
Retired	-	1	2	-	-	-	3
Educational level							
Degree	-	-	1	1	-	-	2
Diploma	1	1	3	-	-	-	5
High school	1	2	-	2	1	1	7
Some school grade	8	5	3	2	3	1	22
Availability of dairy workers							
Satisfactory	7	5	3	4	3	2	24
Moderate	2	2	3	-	1	-	8
Unsatisfactory	1	1	1	1	-	-	4
Common source of information							
Animal health personnel	8	7	6	4	1	2	28
Husbandry professionals	1	1	1	-	-	-	3
Extension agents	1	-	-	1	3	-	5
Availability of extra land							

for animal feed production							
Yes	-	-	2	5	2	-	9
No	10	8	5		2	2	27
Feed stuffs available							
Yes	-	-	-	-	-	-	0
No	10	8	7	5	4	2	36
water source available for							
Yes	10	8	7	5	4	2	36
No	-	-	-	-	-	-	0
Number of times cows are milked							
Once	-	-	-	-	-	-	0
Twice	10	8	7	5	4	2	36
Trice	-	-	-	-	-	-	0
Availability of farm record							
Yes	-	1	6	1	-	1	9
No	10	7	1	4	4	1	27
Exercising animals							
Yes	5	3	3	3	4	1	19
No	5	5	4	2	-	1	17

8. 4. Result of questionnaire survey on farm observation and environmental management

Questions	Urban			Peri-Urban			Total
	Small	Medium	Large	Small	Medium	Large	
Animal house							
Modern	9	5	7	5	1	1	25
Mixture	1	3	--		3	1	8

Ventilation							
Poor	2	4	7	1	1	--	15
Moderate	3	2	--	3	2	2	12
Good	5	2	--	1	1	--	9
Drainage							
	4	4	7	1	2	1	19
Moderate	4	2	--	--	1	1	8
Good	2	2	--	4	1	--	9

8.5. Flow chart for isolation and identification of bacteria from milk

Milk sample collection

▼

CMT

▼

Culture respective of CMT result

▼

Inoculation on blood agar

▼

Incubation at 37^{0}C for 24-48 hours

▼

Observation of colony characteristics

▼

Subculture on blood and macConkey agar

▼

Incubation at 370C for 24-48 hours

▼

Gram stain

▼

Primary identification

- Growth on MacConkey agar
- Catalas test
- Oxidase test
- O-F test
- Motility test

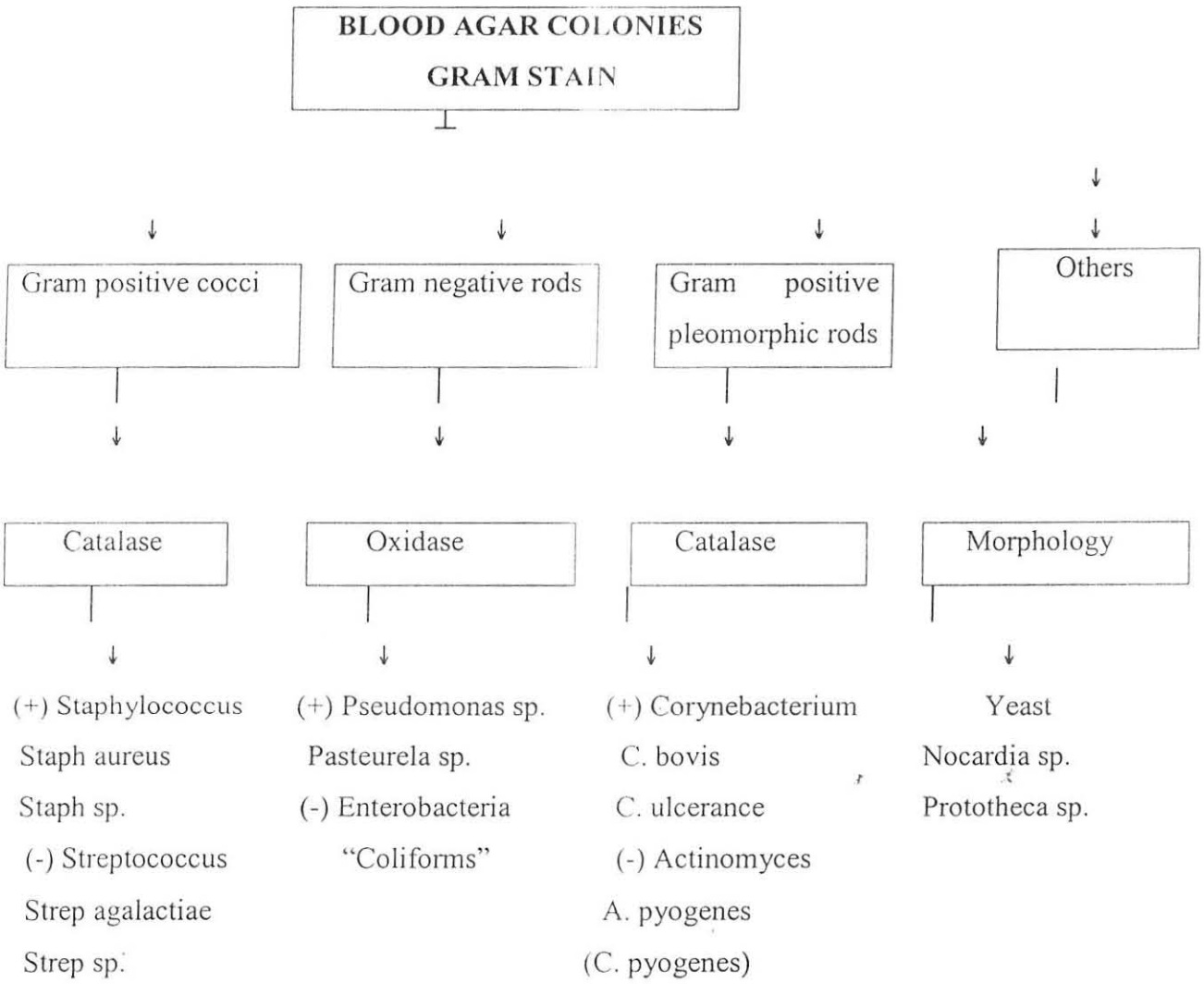
▼

Secondary identification test

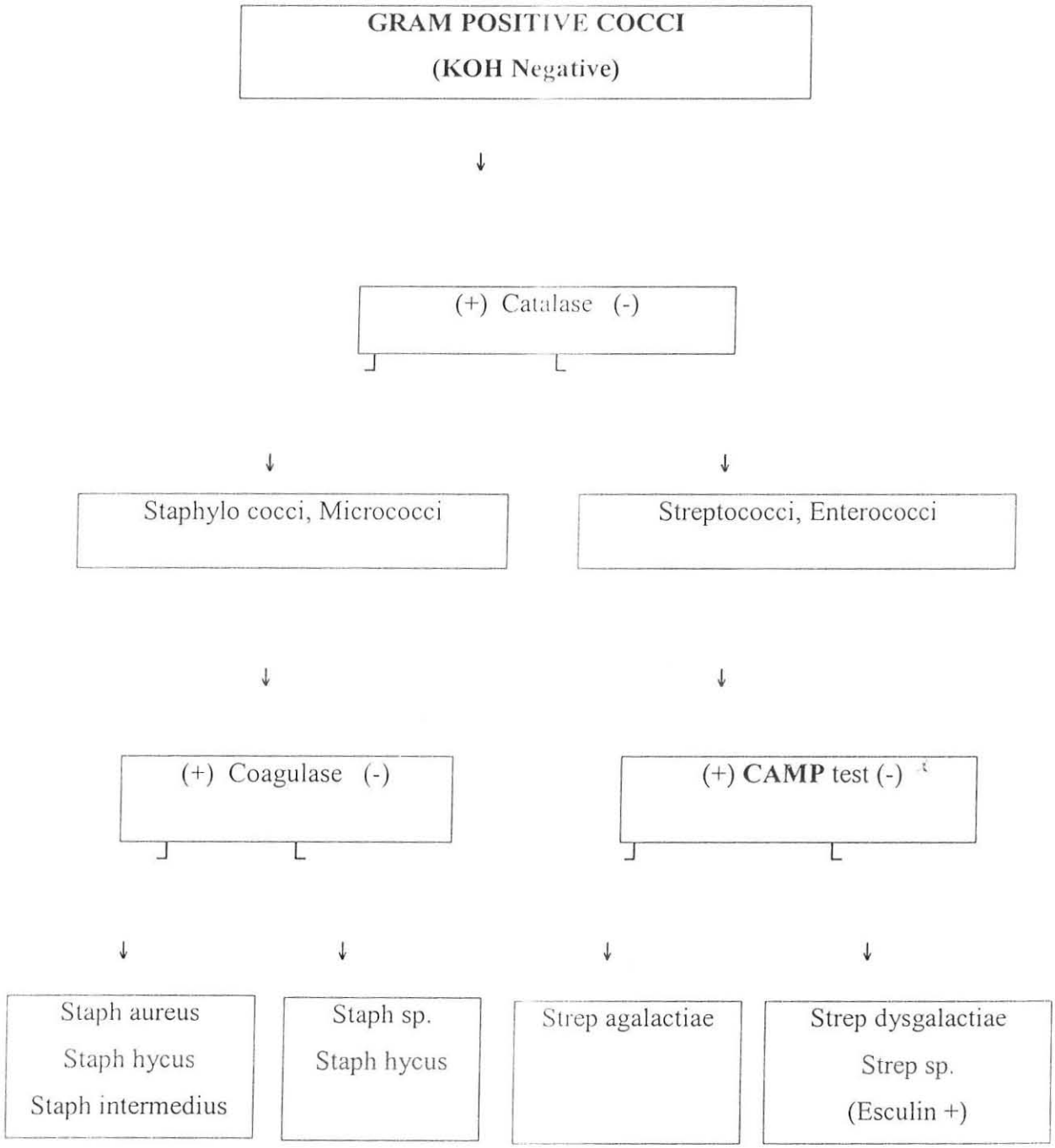
Biochemical tests

Carbohydrate fermentation

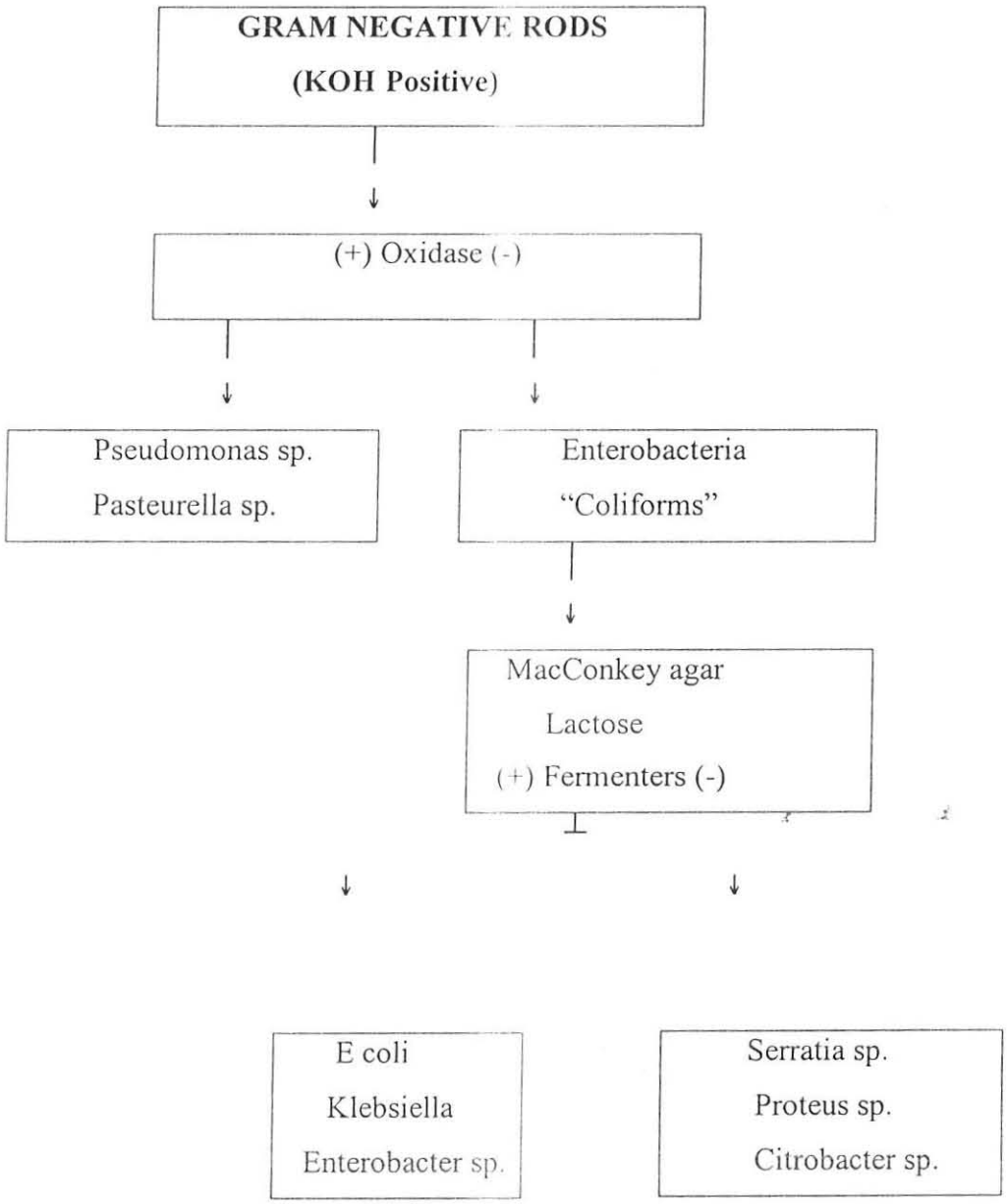
8.6. Microbiological diagnosis for routine culture (source Carter, 1996)



8.7. Microbiological diagnosis for gram-positive organisms



8.8. Microbiological diagnosis for gram-negative organisms



Gram positive pleomorphic rods associated with mastitis

Test	Corynebacterium pyogenes	Actinomyces ulcerance	Corynebacterium bovis
Catalase	-	+	+
Haemolysis	+	+	-
Growth in 9% sodium chloride	-	-	+

8.9. Primary identification test

Gram's stain (Carter, 1996)

Procedure:

Make a thin smear or film

Allow the film to dry in air

Fix the film by passing through yhe bunsen flame several times

Flood the slide with crystal violat for 30 to 60 seconds

Pour off the stain and wash the remaining stain with iodine solution

Wash off the iodine and shake the excess water from the slide

Decolorize with aceton or ethyle alcohole for 30 seconds

Counter stain with carbol fuchsin or safranin for 30 to 60 seconds

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

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

Procedure: a loopfull of bacteril growth is taken from rhe top of the colonies avoiding the blood agar medium. The bacterial cells are placed on a clean microscope slide and a drop of 3%

hydrogen peroxide is added. An effervescence of oxygen gas, with in a few seconds, indicates a positive reaction.

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

Principle: The cytochrome oxidase inzyme is able to oxidize the substrate tetramethyl-p-phenylenediamine dihydrochloride, forming a coloured end product, indophenol.

Procedure: prepare a solution of 1% tetramethyl-p-phenylenediamine dihydrochloride, then a piece of filter paper is moistened in a petri dish with fresh reagent and the test bacterium is streaked firmly across the filter paper with a glass rod. A dark purple colour along the streak line with in 10 seconds indicates the positive reaction. *Pseudomonas aerugenosa* can be used as a positive control organism.

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

Procedure: Prepare O-F base medium and when the O-F base has cooled to 50^0C add 20 ml of strile glucose solution in to 200 ml of O-F base, for a final concentration of 1% glucose and dispense in to tubes. Two tubes of the O-F medium are heated in a beakerof boiling water immediately before use to drive off any disolved oxygen and the tubes are then cooled rapidly under cold running water. Both tubes are stab-inoculated with the bacterium and a layer of sterile paraffin oil is layered on top of one of the tubes (sealed tube) to a depth of about one centimeter and the tubes are incubated at 37^0C and examined in 24 hours and then daily for up to 14 days.

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

Procedure: SIM medium (BBL) is used to detect motility and the medium is stab inoculated using a straight wire. Then the tube is examined for motility after 24 and 48 hours. If there is a diffused growth through out the medium, the bacterium is motile. The growth of none motile bacteriom is confined to stab line. To interprate the resukt, hold the tube against a good light and compare the inoculated tubes with un inoculated one.

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

Procedure: A culture of the staphylococcus aureus with a wide zone of partial haemolysis (beta-haemolysin) is streaked accrose the center of a sheep or ox blood agar plate. Astreak of the suspect Group B *streptococcus* is made at right angle to, and taken to with in 1 to 1.5 mm of staphylococcal streak. The plate is incubated at 37^0C for 18 to 24 hours. An arrow-head of complete haemolysis, indicates a positive CAMP test. The group B *streptococci* produce diffusible metabolites that complete the lysis of the red cells, only partially haemolysed by the beta-haemolysin of the *staphylococcus.*

8.10. Secondary identification test

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

Principle: Indole positive bacteria posses an nzyme tryptophanase which converts tryptophan to indole.

Principle: Stab inoculte SIM medium in a tube with test bacterium and inocubate at 37^0C for 18 to 24 hours then add 0.2 ml kovac's reagentto the medium and stand for 10 minutes.

Interpretation: The formation of datk red ring on the layer 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 otganisms to produce strong acid.

Procedure: Inoculate Glucose phosphate peptone water (MR-VP) broth with pure culture of test organism and incubate at 37^0C for two days, then add drops of MR solution in to the medium.

Interpritation: Production of red colour indicates a positive and yellow negative result.

Voges-proskauer (VP) test (Quinn, *et al.*, 1999)

Principle: Some organisms produce acetoin as a chief product of glucose metabolism and form less quantity of mixed acid.

Procedure: Inoculate MR-VP broth with pure culture of the test organism and incubate at 37^0C for 2 days. Then aliquot 1 ml of broth to a clean test tube and add 0.6 ml of 5% of alphanaphthol in absolute pure ethyl alcohol and then0.2 ml 40% KOH. Shek the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes.

Interpretation: A pink (red) colour indicates a positive reaction and colourless is a negative result/

Ureas Test (Quinn *et. al.*, 1999)

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

Procedure: The surface of the agar slant is streaked with the test organism and incubated at 37^0C for 18 to 24 hours.

Interpretation: Organisms that hydrolyze urea rapidly may produce positive reaction in 1 or 2 hours. Red (pink) colour through out the medium indicates a positive reaction.

8.11. Identification of gram positive cocci (Quinn *et. al.*, 2005)

Organism	Appearance in stained Smears	Coagulase production	Catalase production	Oxidase production	O-F test
Staphylococcus spp	Irregular clusters	±	+	-	F
.Micrococcus spp.	Packets of four	-	+	+	O
Streptococcus and Micrococcus spp.	Chain	-	-	-	F

(+) – Positive, (-) – Negative, (F) – Fermentative, (O) – Oxidative,

(±) – Positive or negative

8.12. Differentiation of mastitis causing *staphylococci spp.*(Quinn *et. al.*, 1999)

Test	*staph. Aureus*	CNS	*Micrrococci*
Catalase	+	+	+
Coagulase	+	-	-
Haemolysis	+	-	-
Manitole (A)	+	-	-
Maltose (A)	+	v	-
Glucose (A)	+	+	-

(+) - Positive reaction, (-) – Negative reaction, (v) – Variable reaction, (A) - Acid

8.13. Differentiation of mastitis causing *streptococci spp.* .(Quinn *et. al.*, 1999)

Species	CAMP test	Growth on MacConky agar	Easculin hydrolysis	Other tests
Strep. Agalactiae	+	-	-	-
Strep. Uberis	±	-	+	Manotol (A)
Strep. Disgalactia -		-	-	Salicin (A)
Enterococcus faecalis (A)	-	+	+	Salicin Manitol (A)
Enterococcus pyogenes	-	-	-	Salicin (-)
Enterococcus pneumonia (-)	-	-	-	Manitol

(+) – Positive, (-) – negative, ± - Negative or positive, (A) - acid

8.14. Growth characteristics and biochemical reactions of members of *Enterobacteriaceae, which* causes mastitis (Quinn *et. al.*, 2005)

Test	Escherchia coli	Yersinia spp.	Proteus Spp.	Enterobacter aerogenes	Klebsiella pneumonia
Clinical importance	Major p/gen	Maj p/gen	Oppor p/gen	Oppor p/gen	Oppor
Culture characteristics	haemo Lytic	- growth	Swarming	Mucoid	Mucoid
Motility at 30^0C	Motile	Motile	Motile	Motile	Non motile
Lactose fermentation	+	-	-	+	+
Indol production	+		v	±	- -
Methyl red test	+	+	+	-	-
Voges proskauer test	-		-	v	+ +
Citrate utilization	-		-	v	+ +

H_2S production	-	-	+	-	-
in TSI agar					
Lysine decarboxylase	+		--	+	+
Urease activity	-		+	+	- +

When cultured on non inhibitory medium

(+) – Positive, (-) – Negative, p/gen – pathogen, (±) – positive or negative,

(v) – reaction varies with individual species

8.15. Differentiation of *corynebacterium* and *actinomyces spp.*

Carter (1994), Quinn *et. al.*, (1999)

Species	Catalase test	Haemolysis	glucose	Lactose	Maltose	trehalose
C. alcerens	+	v	+	-	+	+
C. bovis	+	-	-	-	-	-
C. pseudo						
Tuberculosis	+	+	+	+	+	+
A. pyogenes	-	+	+	+	v	

(v) – variable reaction

8.16. Differentiation of bacillus spp.(Carter, 1994)

Species	Citrate	Arabinose	Manitol	Voges proskauer
Bacillus cerus	+	-	-	+
Bacillus pumilus	+	+	+	+
Bacillus brevisd	-	d	-	-
Bacillus coagulase	d	d	d	d

d - 11-89% strains are positive

8.17. Media used for isolation and identification of bacteria

1. Blood agar base (BBL*, Becton Dickinson, USA)

Composition (g/l): Heart muscle, infusion from(solids) 2.0; pancreatic digest of cascin 13.0; Yeast extract 5.0; sodium chloride 5.0; agar 15.0

Preparetion: Suspend 40.0g of the powder in 1 liter of distilled water. Mix throughly heat with frequent agitation and boil for 1 minute to completely disolve the powder. Autoclave at 121^{0}C for 15 minutes. Cool the base to 45 to 50^{0}C and add 5% sterile defibrinated sheep or ox blood.

2. MacConkey agar (Oxoid, Hampshire, England)

Composition (g/l): Peptone 20.0; lactose 10.0; bile salt No. 3 1.5; sodium chloride 5.0; neutral red 0.03; crystal violate 0.001; agar 15.0

Preparation: Suspend 51.5g in 1 liter of distilled water. Bring to boil completely sterilize by autoclaveing at 121^{0}C for 15 minutes.

3. SIM medium (BBL*, Becton Dickinson, USA)

Composition (g/l) Pancreatic digest of casecin 20.0; peptic digest of animal tissue 6.1; ferrous amonium sulfate 0.2; agar 3.5

Preparation: Suspend 30g of the powder in 1 liter distilled water. Mix throughly, heat wit frequent agitation and boil for 1 minute, Autoclave at 121^{0}C for 15 minutes.

4. O-F basal medium (Merc, Darmstadt, Germany)

Composition (g/l): Peptone from cascin2.0; yeast extract1.0; sodium chloride 5.0; D-potasium hydrgen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5

Preparatio: suspen 11g in 1 liter of distilled water by heating in a boiling water bath or in a current steam, autoclave for 15 minutes at 121^{0}C. at approximately 50^{0}C mix in 100ml liter filter

sterilized 10% solution of D(+) glucose, lactose, sucrose, or other carbohydrates; dispense in to tubes to give a depth of approximately 3cm.

5. Phenol-red broth base (Merc, Darmstadt, Germany)

Composition (g/l): peptone from casein 5.0; pepton from meat 5.0; sodium chloride 5.0; phenol red 0.018.

Preparation: Suspend 15g in 1 liter of distilledwater; dispense in to tubes and insert fermentation tubes; if necessary; autoclave for 15 minutes at 121^0C At less than 60^0C add the reactants (final concentration 5 to 10g/liter) as sterlie solution.

6. Manitol salt agar (difco, Detroit, USA)

Composition (g/l): peptose pepton No.3 10.0; Bacto-beef extract 1.0; -manitol 10.0; sodium chloride 75.0; Bacto-agar 15.0; phenol red 0.025

Preparation: Suspend 111g in 1 liter distilled water and heat to boiling to dissolve completely. Sterilize with an autoclave for 15 minutes at 121^0C/15 pound presure. Cool to 45 to 50^0C and dispense in to petri dish.

7. Nutrient agar (Oxoid, Hampshire, England)

Composition (g/L): "Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0' agar 15.0.

Preparation: Suspend 28g in 1 liter of distilled water. Bring to boil to disolve completely, autoclave at 121^0C for 15 minutes.

8. Triple Sugar Iron agar (TSI) (marck, Germany)

Composition (g/l); Pepton from cascin 15.0; meat extract 3.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; D (+) glucose 10.0; ammonium iron (III) citrate 0.5; sodium thiosulfate 0.5; phenol red 0.0024; agar-agar 12.0.

Preparation: Suspend 65 g in 1 liter of istilled water by heating in a boiling water bath or in a current steam. Dispense in to tubes, autoclave for 15 minutes at 121^0C. Allow to solidify to give agar slant.

9. Simmons citrate agar (BBL*, Becton Dickinson, USA)

Composition (g/l): Ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.0; sodium chloride 5.0; sodium citrate 2.0; magnisium sulphate 0.2; agar 15.0; bromothymol blue 0.08.

Preparation: Suspend 24.2 g of the powder in 1 liter of distilled water. Mix throughly, heat with frequent agitation and boil for 1 minute to completely disolve the powder. Autoclave at 121^0C for 15 minutes. Cool tubed medium in a slant position.

10. Edwards medium, modified (Oxoid, Hampshire, England)

Composition (g/l); "Lab-lemco" powder 10.0; peptone 10.0; aesculine 1.0; sodium chloride 5.0; crystal violate 0.0013; thallus sulphate 0.33; agar 15.0.

Preparation: Suspand 41g in 1 liter of distilled water. Bring to the boil to disolve completely. Sterilize by autoclaving at 121^0C for 20 minutes. Cool to 50^0C, add 5 to 7% sterile sheeo or ox blood. Mix well and pour in the petridish.

11. Eosin methylin blue agar (Modifid) Levine Oxoid, hampshire, England)

Composition (g/l): Poptone 10.0; Lactose 10.0; dipotassium hydrogen phosphate 2.0; eosine y 0.4; methylen blue 0.065; agar 15.0.

Preparation: Suspend 37.5g in 1 liter of distilled water. Bring to the boile to disolve completely. Sterilise by autoclaving at 121^{0}C for 15 minutes. Cool to 60^{0}C and shake the medium in order to oxidise the methylen blue (i.e. restore it's blue colour and to suspend the precipitate which is an essential part of the medium.

12. MR-VP medium (oxoide, hampshire, England)

Compositin (g/l): Peptone 7.0; glucose 5.0; phosphate buffer 5.0.
Preparation: suspand 17.0g in 1 liter of distilled water, mix well, distribute in to tubes and autoclave at 121^{0}C for 15 minutes.

13. Urea agar base (BBL*, Becton Dickinson, USA)

Composition (g/l): Pancriatic digest of gelatin 1.0; dextrose 1.0; sodium chloride 5.0; potassium phosphate 2.0; urea 20.0; phenol red 0.012.

Preparation: Suspend 20.0g of powder in 100 ml of distilled water, mix throughly sterilize by filtration. Suspend 15g if agar in 900 ml distilled water, and autoclave at 121^{0}C for 15 minutes. Cool to 50^{0}C and add the 100 ml prepared urea agar base. Mix throughly and dispense aseptically in sterile tubes. Cool tube medium in a slant position so that deep butts are formed.

14. Muller Hinton agar (BBL*, Becton Dickinson, USA)

Composition (per liter-purified water): Beef extract 2gm, acid hydrolysate of casein 17.5; Starch 1.5, agar 17.0.

Preparation: Suspend 38g of the powder in 1 liter of distilled water, mix throughly. Heat with frequent agitation and boil for 1 minute. Autoclave at 121^{0}C for 15 minutes.

8.18. Procedure to conduct antibiotic susceptibility test (Quinn, *et al.*, 1999)

Preparation of the inoculum

Inoculate a distinct colony in to 5 ml of nutrient broth, and incubate at 35 to 37^0C for about 5 hours. Then the turbidity is compared with 0.5 MacFarnand standard. This standard is prepared by adding 0.5 ml of 1% (11.75g/liter) $BaCl_2 2H_20$ to 99.5 ml of 1% (0.36N0 H_2So4

Inoculation to Mueller Hinton agar

Mueller Hinton agar autoclaved at 121^0C for 15 minutes is cooled to 50^0C and poured in to a sterile petridish on a level surface to a depth of 4 mm; this is equivalent to 60 ml in a 15 cm plate and 25 ml in a 10 cm plate. For slow growing bacteria 5% defibrinated whole blood could be added. Then a sterile cotton swab on a wooden applicator stick is used to transfer the diluted bacterial suspension to a plate, excess fluid must be squised out by the rotating the swab against the sides of the tube. The plate is seeded uniformly by rubbing the swab against the entire agar surface in to three different plans roughly 60 degrees to each other.

Disk application

With in 15 minutes (time used to dry the inoculums) after the plates are inoculated, antibiotic impregnated discs are applied to the surface of the inoculated plates by using a sterile forceps. All discs are gently pressed down on to the agar with forceps to ensure complete contact with the agar surface. The discs should not be closer than 1.5 cm to the edge of the plate and they should rest 3 cm apart from each other. The large petri dishes can accommodate 9 discs in outer ring and 3 in the center, where as no more than 8 should be placed in a small (10 cm) plates.

Inoculation

Incubate the plates invented aerobically for 16 to 18 hours at 35^0C but not at 37^0C.

Interpretation

Growth inhibition zone is measured in millimeters using a transparent ruler on the under surface of the petridish. For measuring purpose the end point is taken as complete inhibition of growth as determined by naked eye. The result is interpreted according to the table presented bellow.

Zone size interpretive for antimicrobial inhibition zone diameter (mm)

Antimicrobial agents	Disc potency	Resistance	Intermediate	Susceptible
Streptomycin S10	10μg	≤11	12-14	≥15
Oxitetracyclin OXTE 30	30μg	≤14	15-18	≥19
Erythromycin E10	15μg	≤13	14-17	≥18
Pencillin G10 for *staphylococcus*	10IU	≤20	21-28	≥29
Pencillin for other Microorganisms	10IU	≤11	12-21	≥22
Sulphanomide S300	300μg	≤8	9-11	≥12
Polymixin BPB30	300μg			
Kanamycin K30	30μg	≤13	14-17	≥18

4. Membership

Ethiopian Veterinary Association

5. Publication

1. DVM, Thesis, Ovine Moneziosis and Chemo Prophylactic Control Measures. Faculty of Veterinary Medicine, Kishinev Agricultural Institute, 1989, Moldavia (USSR)

2. A review on Etiology and Control of Bovine Mastitis. May 2009, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia

3. MSc, Thesis, Cross-sectional Study of Bovine Mastitis in urban and pri-urban dairy farms of Harar town June, 2010

SIGNED STATEMENT DECLARATION SHEET

"This thesis 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 Ketema Bogale Gelete

Signature _____

Date of submission _____

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

Dr. Yasmin Jibril _____

Dr. Yilkal Asfaw _____

Dr. Tefera Yilma _____