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STUDY ON BOVINE MASTITIS: PREVALENCE, BACTERIOLOGICAL EXAMINATION AND ASSESSMENT OF MILK LOSS IN DURAME TOWN AND ITS SURROUNDING DISTRICT, SOUTHERN ETHIOPIA

MASTER OF SCIENCE IN VETERINARY EPIDEMIOLOGY

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Study on Bovine Mastitis: Prevalence, Bacteriological Examination and Assessment of Milk Loss in Durame Town and Its Surrounding District, Southern Ethiopia

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Submitted to School of Graduate Studies Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine, in Partial fulfillment of The Requirements for the Degree of Master of Science in Veterinary Epidemiology

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisors. Hence, I hereby kindly request the Department to allow me to present the finding of my work and submit the thesis.

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DEDICATION

I dedicate this piece of work to my husband, Mr. Wondimagegn Abosse for his presentable child raise and endless moral and financial support and my daughter and son, Hulubante Wondimagegn and Yesilaseneh Wondimagegn wait me patiently by longing until the end of the thesis work.

STATEMENT OF THE AUTHOR

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

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BIOGRAPHICAL SKETCH

Bayush Tesfaye was born on April 12, 1977 E.C in SNNPR, Kambata Tembaro zone, Kacha Bira Wereda from her mother Mrs. Wudenesh Asfawu and Father Mr. Tesfaye Ayele. She was attends her Elementary Education from1984 E.C to 1987 E.C in Eluababor zone Toba wereda and from 1988 to 1989 E.C in Shinshicho kera Elementary school and attended her junior and Secondary Schools in Shinshico Senior and Secondary School from 1990 to 1993 E.C and she completed her preparatory education in Durame preparatory High school From 1994 to 1995 E.C.

She started her University Education in Jimma University College of Agriculture and school of Veterinary Medicine in September 1996 E.C and graduated with doctor of veterinary medicine (DVM) on June 7, 2000 E.C. After her DVM study, she was employed by Kacha Bira wereda animal health clinic staff and team leader from July 2000 to November 2003 E.C for 2 year and 5 month work experience. After wereda clinic work experience join with different department of zone administration and livestock and fishery department head from November 2003 to 2009 E.C. After 8 years work experience ,in September, 2010, she joined Jimma University College of Agriculture and School of Veterinary Medicine to pursue her post graduate study in veterinary epidemiology specializing.

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LIST OF ABBREVIATIONS

CSA……Central Statistical Authority

SCC……Somatic cell count

CM……..Clinical Mastitis

SCM……Sub Clinical Mastitis

CMT……California Mastitis Test

NMC……...National Mastitis Council Guidelines

SNNPR……...Southern Nation Nationality and Peoples Region

TSI……...Triple Sugar Iron Test

CAMP……..Christie–Atkins–Munch-Petersen

CNS……..Coagulase Negative Staphylococci

KOH…….Potassium Hydroxide

NCCLS…….National Committee for Clinical Laboratory Standard

SPSS…….Statistical Package for Social Sciences

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ABSTRACT

Mastitis is a multifaceted dairy cattle disease with multiple causative agents and economic loss. Cross-sectional study on bovine mastitis prevalence and subclinical mastitis milk yield loss in Durame town and its surrounding district was conducted from January to September 2019 to estimate the prevalence, milk yield losses, associated risk factors and antimicrobial susceptibility testing of the isolates. Californian Mastitis Test, bacteriological culturing, antimicrobial susceptibility test and split-udder trial was conducted to determine production losses. Out of 384 lactating dairy cows were selected from the sampling frame list picked randomly by the simple random sampling method, a total 220 (57.3%) cows were found positive for mastitis: 2.1% clinical and 55.2% sub-clinical. The prevalence in Durame and its surrounding were 78.2% and 53.8%, respectively. Among assessed risk factors, mastitis was 2.5 times higher in the crossbred than local. Prevalence was 2.6 times higher in cows having 6 or more compared to cows having 1–3 calving, Cows in the early lactation were more likely to have mastitis than cows in the late lactation stage. Cows in herds without bedding material were more likely to have mastitis than those cows in farms with bedding material. Cows in farms that did not milk mastitic cows last were more likely to have mastitis than those cows in herds that practice milking mastitic cow at last. Herds feeding their cows before milking were 5.128 times more likely to have mastitis than herds feeding their cows after milking, house floor with non-concrete and barn cleaning once were more likely associated with the udder infection. The most common bacterial isolates were *Coagulase-negative Staphylococci* (35.5%), *Staphylococcus aureus* (30.5%), *Streptococcus agalactia* (15%), *E. coli* (11.6%), *Staphylococcus intermidus* (1.8%) and *streptococcus uberis* (4.3%). The majority of isolates were highly sensitive to Gentamycin (98.3%) and resistant to Polymixyn. The mean milk yield for uninfected healthy quarters was 0.995kg per milking and the rates of milk reduction in infected quarters were 6.2%, 24%, and 50.5%. Quarters with Californian Mastitis Test trace and 1 were combined and considered negligible. Subclinical mastitis lost an average of 22% of its milk production and causes an estimated total loss for each cow per lactation was 2884.59 Eth Birr. Reducing risk factors, early diagnosis and regular screening of cows together with the proper therapeutic management are important to reduce mastitis.

Keywords: *Bovine mastitis, Durame, loss, milk yield, prevalence,*

1. INTRODUCTION

The Ethiopian cattle population is estimated to be about 59.5 million. Out of this total cattle population, the female cattle constitute about 55.5 percent (CSA, 2017). However, milk production often does not satisfy the country's requirements due to different factors.

Mastitis is an inflammation of the udder, a common disease among dairy cows worldwide caused mainly by a bacterial infection (Ruegg *et al*., 2014). It is divided into clinical mastitis (CM) and subclinical mastitis (SCM). Clinical mastitis is known with visible changes in milk and udder while subclinical mastitis is an increased number of inflammatory cells in the milk without an abnormal appearance of the milk or the udder (Lundberg, 2015). Bovine mastitis is an economically important disease due to its impact on the quantity and quality of milk production (FAO, 2015). Mastitis is a complex disease that interacts with microorganisms, host and the environmental factors (Radostits *et al*., 2007). Mastitis causes direct losses due to discarded milk, the cost of medicines and labor cost and indirect costs, loss of future production and increased culling (Huijps *et al*., 2008).

Prevalence of mastitis in Ethiopia and other African regions may impose substantial costs due to direct and indirect losses (Petrovski *et al*., 2006). Ethiopian crossbred dairy cows have an economic loss due to subclinical mastitis. However, most dairy farmers in the country normally do not recognize subclinical mastitis, which incidentally occurs at a much higher frequency than clinical mastitis, while quite few ignore the disease (Mungube *et al*., 2005).

Methods commonly employed for diagnosis of mastitis are screening tests, bacteriological examination and physical examination (Radostits *et al*., 2007). Mastitis treatment can be administered by different routes by intramammary antimicrobials infused into the udder through the teat canal and parenteral treatment given by injection (Pol and Ruegg, 2007; Blowey and Edmondson, 2010).

Application of hygienic measures during milk collection by washing dirty teats and udders aseptically and dried thoroughly before proceeding to sample collection, using milking machines, lactation and dry cow therapy, teat sealers, dietary supplements and culling are used to reduce the incidence of mastitis (Tiwari *et al*., 2013).

Although the disease has been reported by several authors in different parts of Ethiopia (Lakew *et al*., 2009). Available information on the magnitude of sub clinical mastitis economic loss and risk factors of the disease is still scanty. Therefore, the objectives of the study were.

General objective

1. To generate information on the status of the overall prevalence of Bovine clinical and subclinical mastitis risk factors, to estimate milk yield loss due to sub clinical mastitis and to isolate causative agents in lactating dairy cows in the study area.

Specific objectives

1. To estimate prevalence of mastitis and assess associated risk factor
2. To isolate major bacterial species and do antimicrobial susceptibility test of bovine mastitis
3. To estimate milk yield loss due to sub clinical mastitis

1.1. Statement of the Problem

In Ethiopia mastitis has long been known and reported by several authors in different parts of the country (Mungube *et al*., 2005; Mekibib *et al*., 2010 and Megersa *et al*., 2010). However, information on the economic loss of mastitis, risk factors and overall prevalence is inadequate in many areas of the country. Very limited published data are available to quantify production losses and expenditures related to mastitis in developing countries, and thus to assess the economic impact of the disease. Because production systems, environment, management and breeds are different, it is not possible to compare data from developed and developing countries. So there is the need to assess the extent of financial losses due to mastitis on the basis of studies conducted in the developing countries (FAO, 2014). Economic loss in Ethiopian highland crossbred dairy cows is due to subclinical mastitis. Such information is important to envisage when designing

appropriate strategies that would help to reduce its prevalence and effects (Megersa *et al.*, 2010; Mekibib *et al.*, 2010).This situation is also true in the current study area Durame town and its surrounding although the area is known with high crossbred dairy population and dairy farming with increasing milk production. Yet, adequate information on the prevalence and economic loss of subclinical mastitis in the area is unknown except for few fragments of information from reports on cases of clinical mastitis that has been presented to the veterinary clinic for treatment. Therefore, this study fills the information gaps related with prevalence of mastitis, bacterial species of bovine mastitis and milk yield loss of sub clinical mastitis and helps to forward practical recommendation based on the findings.

2. LITERATURE REVIEW

2.1 Definition of Bovine Mastitis

Mastitis is defined as an inflammation in the mammary gland most commonly caused by a bacterial infection, but other origins, such as yeasts, fungi, algae and trauma may also result in mastitis (Ruegg *et al*., 2014). Mastitis is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue of the udder and affects the quality and quantity of milk (Sharma *et al.*, 2011). Consequences of mastitis reduces milk yield, increases culling rates, bring treatment costs and occasional death from severe infections. In addition, some udder pathogens affect food safety because they produce toxins that cause food poisoning, as in the case of *Staphylococcus aureus* (Rosec *et al*., 1997).

Mastitis can be classified into two main categories, subclinical and clinical. Subclinical mastitis is defined by an increased number of inflammatory cells in the milk without an abnormal appearance of either the milk or the udder. Clinical mastitis is palpable or visible changes in milk and udder and can be mild only abnormalities in the milk, moderate clinical inflammatory signs of the udder tissue or severe additional systemic symptoms (Ruegg *et al*., 2014).

2.2 Etiologic Agents and Source of Infection

To date, more than 140 potentially pathogenic species that cause bovine mastitis (Petrovski*et al*., 2011). Based on the pathogen involved the disease is broadly classified into four types such as bacterial, mycotic/Fungal/algal, Mycoplasmal and Nocardial mastitis. The viruses have least clinical significance (Shaheen *et al*., 2016).

Bacterial mastitis are broadly two type: - Gram-positive and Gram-negative, of which the major agents of mastitis are the Gram-positive bacteria including *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma bovis* (Owens *et al*., 2000).The chief agents of gram negative mastitis include *E.coli*, *Proteus* and *Klebsiella* species (Menzies *et al*., 2003).

Fungal infection of bovine mammary tissue caused by contamination of teat dips, intramammary infusions and moldy surroundings play significant role. The important

mycotic mastitis causes are *Aspergillus fumigatus* and *Candida albicans* (Radostits *et al*., 2006).

Algal agent like *Protothecazopfii* is also incriminated in bovine mastitis, as a result of algal contamination of feed and fodder, drinking water, and cattle premises by house hold sewage, discarded food items including bread, rotten vegetables, and fruits. The disease is more prevalent in the regions where cattle are often grazed in the vicinity of public parks, lakes and tourist places (Marques *et al*., 2008).

Among several species *Mycoplasma bovis* and to some extent *Mycoplasma bovirhinis* and possibly to a lesser extent *Mycoplasmal canadense* are causal agents of contagious bovine mastitis (Shaheen*et al*., 2016). Nocardial mastitis is saprophytic bacteria in origin; the causal agents, *Nocardia asteroids*, *Nocardia braziliensis* and *Nocardia farcinicus* are involved in several chronic and granulomatous forms of mastitis. Bovine mastitis due to nocardia occurs as a result of poor environmental hygienic conditions, soil contamination of udders, teat dips and intramammary infusions (Ribeiro *et al*., 2007).Mastitis is epidemiologically categorized in to contagious and environmental mastitis (Cervinkova *et al*., 2013). Both of which severely damage the udder tissue of affected cows (Bradely, 2002).

The viruses have least clinical significance. The main viral affections of the bovine udder are ulcerative bovine mammilitis (mammary pustular dermatitis) due to bovine herpes virus, pseudo cowpox (milker's nodule) and cow pox viruses, which are truly the infection of epidermis/dermis of udder (Shaheen *et al*., 2016).

2.2.1. Contagious Mastitis Pathogens

Contagious mastitis is caused by pathogens live and multiply on and in the cow's mammary gland and are spread from cow to cow, primarily during milking. Contagious pathogens include *Staphylococcus aureus, Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* (Radostis *et al.,* 2000).

2.2.2. Environmental Mastitis Pathogen

Environmental mastitis is caused by pathogens found in the habitat of the cow, such as soil, plant material, manure, bedding, or a contaminated water source. Frequently, isolated causative pathogens that contribute to environmental bovine mastitis include members of streptococci and gram-negative bacteria, such as *Escherichia coli* and *Klebsiella* (Carrillo and Miranda, 2012).

2.3. Bovine Mastitis Etiologic Agent Occurrence in Ethiopia

In Ethiopia, about bovine mastitis pathogens, several studies have been carried out from milk samples. The results of these studies are summarized in Table 1. *Staphylococci* have been found to be the most common mastitis pathogen in Ethiopia (Mekonnen *et al*., 2005; Almaw *et al*., 2008; Lakew *et al*., 2009). *Staphylococcus aureus* is cultured from milk samples from both CM and SCM cases, whereas CNS is predominantly cultured from SCM cases (Mekonnen *et al.*, 2005; Lakew *et al*., 2009).

Table 1.Identified Contagious and Environmental Pathogens of Bovine mastitis in Different Parts of Ethiopia

Bacteria	Authors and areas of studies in Percentile								
	Fentahune *etal*. (2018)	Tekle & Berihe (2016)	Sarba & Tola (2017)	Asmare & Kassa (2017)	Duguma *et al.* (2014)	Mekonnen (2018)	Belachew (2017)	Girma *et al.* (2012)	Kedir *et al*. (2016)
	Tigray region	SNNPR	Oromia region	SNNPR	Oromia region	Amhara region	Addis Ababa	Harerghe	Dire Dawa
S. aureus	31.2	21.1	30.93	39	43.3	9	46.63	35.5	48.4
CNS				18.6	3.9	31	13.47		34.4
S. epidermidis		13.5							
S. hyicus		7.7						4.9	4.5
S. agalactiae			5.15		12.2		2.1	19.9	
S. uberis			12.37		2.8		5.9	5.8	
S. dysgalactiae			5.15		7.2			5.8	
S. intermedius		17.3					3.7	6.6	12.7
E. coli	25.7	3.8	6.18	13.6			2.1	5.8	
A. pyogenes					3.9				
B. cereus		3.8					3.7	0.8	
C. bovis			13.4		3.3			4.1	
Micrococcus spp.		9.6			17.2		5.9		
Klebsiella spp.	5.8	13.5						2.5	

2.4. Spread of Infection

Infection enters by way of the teat and can spread from cow to cow by milkers' hands or the cups of the machine, in a heavily infected herd the skin of the cows' bodies, milkers' clothes, floor, partitions and less easily by towels. In an infected herd, a large proportion of

organisms hiding and these may be a source of infection of the udder itself in the same cow or in another. The skin of the teats and the milkers' hands may remain infected from one milking to another (Edward, 2005).

2.5. Factors that Affect Occurrence of Bovine Mastitis

Bovine mastitis is predisposed by several epidemiological risk factors that play significant role in causing mammary incompetence to protect it from the invasion of infectious agents. The risk factors include the host factors, environmental factors and the pathogen factors (Shaheen *et al*., 2016).

2.5.1. Host Risk Factor

A great number of cow-specific risk factors for CM have been identified, including breed, parity, period of lactation, udder and teat morphology, age, milk production and number of milk somatic cells increase (Peeler *et al*., 2000; Nyman *et al*.,2007; Valde*et al*., 2007). The levels of SCC are elevated in early lactation and gradually increase towards the end of lactation (Schepers *et al*., 1997). Early stage and late stage of the mammary glands were the most susceptible stages. This is possibly due to absence of dry cow therapy that is considered major factor contributing to high prevalence at early lactation (Biffa *et al*., 2005).

Prevalence of mastitis is highest in pure breeds followed by crosses; and indigenous zebu being less frequently affected than others. The increase in prevalence in exotic breeds as opposed to local indigenous zebus could be the indigenous zebu are low in milk production and Higher yielding cows are more susceptible to mastitis (Radostits *et al*., 2006).

Age of cows has effects in occurring of mastitis. It has been shown that manifestation of mastitis in infected quarter’s increases with advancement of age in cows (Harmon *et al*., 1994). This may be due to more dilated teat canals in older age, permanent udder tissue damage resulting from the primary infection or due to an increased cellular response to intra mammary infection after parturition, early lactation and during the dry period and the incidence of mastitis is reported to be higher during these times(Sharma *et al*., 2011).

The prevalence of SCM increases with increasing lactation number and parities (Dego and Tareke, 2003; Mungube *et al.,* 2004; Joshi and Gokale, 2006; Rahman *et al.,* 2009; Lakew *et al*., 2009; Awale *et al.,* 2012; Hameed*et al.,* 2012; Girma *et al.,* 2012; Moges *et al.,* 2012; Jarassaeng *et al.,* 2012; Islam *et al.,* 2011). Cows with the most pendulous quarters appear to be the most susceptible to mammary infections, the pendulous udder exposes the teat and udder to injury and pathogens easily adhere to the teat and gain access to the gland tissue (Almaw, 2004; Sori *et al*., 2005).

2.5.2 Environmental and Pathogen Risk Factors

The cows' environment influences the number and types of bacteria exposed to their ability to resist those organisms. The design of housing system, hygiene, and size of milking cow herd, milking practice and the climate interact to influence the degree of exposure of a cow to mastitis pathogens (Radostits *et al.,* 2006). Moisture, mud and manure present in the environment of the animals are primary sources of exposure for environmental mastitis pathogens. In fact in many studies in Ethiopia such as those conducted by Dego and Tareke, 2003; Lakew *et al*., 2009, a higher prevalence is recorded in cows with poor hygiene in the milking process. Intensively managed cows present a higher risk for the development of mastitis, followed by semi-intensive, with least risk among extensively managed animals (Sori *et al*., 2005).

The occurrence of mastitis varies from season to season, because growth and multiplication of organisms depends on specific temperature and humidity. Incorrect ventilation, with high temperature and relative humidity, encourages the multiplication of various bacteria. Exposure of animals to high temperature can increase the stress of the animal and alter immune functions (Sudhan and Sharma, 2010). In Ethiopia, it was noticed by Dego and Tareke, 2003 that the prevalence was higher in the rainy season than in the dry season. Different types of milking methods (stripping, knuckling, full hand method, machine milking) are practiced by dairy farmers. Faulty milking practices, especially knuckling, cause great harm to tissue and they become prone to infection (Sudhan and Sharma, 2010). Summarize different risk factors in the following Table 2.

Table 2Main Factors Identified as a Risk for the Occurrence of the Bovine Mastitis

Risk factors	Occurrence of Mastitis	Source
Production level	Higher in high yielding bovines Holstein Friesian , Jersey and crossbred dairy cows	Moges *et al.*, 2012; Sudhan and Sharma, 2010; Sori *et al*., 2005; Lakew *et al*., 2009
Quarters appearance	Cows with pendulous quarters appear to be the most susceptible to mammary infections	Almaw, 2004; Sori *et al*., 2005
Teat size	Long teats increase the risk of accidental trauma	Almaw, 2004
Breed	Prevalence is highest in pure breeds followed by crosses and indigenous zebu.	Radostits *et al*., 2006
Age, lactation number & parities	Prevalence of SCM increases with age, increasing lactation number and parities. Higher prevalence of mastitis in older animals.	Dego and Tareke, 2003; Awale*et al*., 2012; Moges *et al*., 2012; Lakew *et al*., 2009 , Girma *et al.*, 2012
Seasonality	Prevalence was higher in the rainy season than in the dry season	Dego and Tareke, 2003; Tilahun and Aylate, 2015
Milking methods	Faulty milking practices, especially knuckling, cause great harm to tissue and they become prone to infection. Higher prevalence of the disease in animals milked by folded thumb	Sudhan and Sharma,2010, Awal *et al*., 2012
Calf suckling	Highest prevalence of mastitis in animals with calf suckling	Hameed *et al.*, 2012
Moisture, mud and manure in the environment	Moisture, mud and manure present in the environment of the animals are sources of exposure for mastitis pathogens	Sudhan and Sharma, 2010
Poor hygiene in the milking process.	Higher prevalence is recorded in cows with poor hygiene in the milking process.	Lakew *et al*., 2009; Dego and Tareke, 2003
Management system	Intensively managed cows present a higher risk followed by semi-intensive with least risk	Sori *et al*., 2005
Housing systems	Mastitis prevalence increases in herds housed under poor stable and drainage conditions	Sudhan and Sharma, 2010
Weather & climate	A higher incidence of mastitis during summer rainy months	Akyuz *et al*., 2010 and Godden *et al*., 2003

2.6. Economic Impact of Mastitis

Mastitis, both clinical and subclinical is known for resulting in a substantial economic loss. Milk yield loss, loss from discarded milk, veterinary service, medicine, increased sanitation, additional labor and equipment major economic loss. Subclinical mastitis in cattle is estimated to result in a loss of 1592.87 Indian rupees (INR). The largest loss was due to milk yield loss and medicine (Singh *et al*., 2014) direct losses due to clinical mastitis in cows to be 2086.96 INR per clinical case (Christy, 2014 ; Halasa *et al*., 2007) also mention a poorer product quality and culling of diseased animals as factors that affect the economy. Subclinical mastitis (SCM) is of great economic importance to dairy farmers because it results in reductions in milk yield and undesirable changes in the milk's composition (Seegers *et al*., 2003 and Halasa *et al*., 2009).

Very limited published data are available to quantify production losses and expenditures related to mastitis in developing countries, and thus to assess the economic impact of the disease. Because production systems, environment, management and breeds are different, it is not possible to compare data from developed and developing countries. So there is the need to assess the extent of financial losses due to mastitis on the basis of studies conducted in the developing countries (FAO, 2014).It is important to bear in mind that mastitis cows are a constant source of contagion due to shedding of bacteria (Halasa *et al*., 2007). What farmers may not notice and may not be aware of is the indirect cost stemming from reduced reproductive performance. Studies confirm that mastitis has detrimental effects on reproductive efficiency of dairy cows and thus negatively affects the profitability of dairy herds (Ahmadzadeh *et al*., 2010).

In Ethiopia total milk production losses accounted for 78% caused by mastitis (Schepers and Dijkhuizen, 1991). Bovine mastitis causes annual production losses of 22.3% in cross breed cows and 2.24% in local breed lactating cows in one year production cycle in an animal production and research center and small holder dairy farms in horoguduru wollega zone, western Ethiopia (Beyene *et al*.,2017).

In the split-udder trial investigation, milk production was reduced by 1.2%, 6.3% and 33% in quarters with CMT score 1+, 2+, & 3+, respectively in central highland of Ethiopia (Mungube *et al*., 2005) and 1+ (25%), 2+ (34%) and 3+ (48%)production loss in Debre Zeit dairy farms (Tesfaye *et al*., 2010). In the crossbred dairy cows under Ethiopian highland conditions, the split udder trial showed that a quarter with subclinical mastitis lost on average 17.2% of the potential milk production (Mungube *et al*., 2005). The quarters with S. aureus SCM lost on average 34.5% of the potential milk production (Tesfaye *et al*., 2010).

The economic loss from mastitis in the urban and peri urban area of Addis Ababa is U$ 58 and 78.65 per cow per lactation, respectively. Losses were highest in large-scale (13%) farms and lowest (3.7%) in small-scale and overall financial loss for each cow per lactation was 984.64 Eth Birr (US$78.65) and losses in large farms 1,882.40 Eth Birr or US$150.35 (Mungube *et al*., 2005 and Tesfaye *et al*., 2010).

2.7. Public Health Significance

Milk is a well-known medium that favors the growth of several microorganisms. Milk from a sub clinically mastitis cow commonly contains the etiological agents, while milk from non-mastitis cows is known to be often contaminated from extraneous dirt or unclean processing water. The health hazards posed by milk-borne zoonotic diseases such as brucellosis, tuberculosis and mastitis-related enterotoxaemia are well-documented (Franz *et al*., 1999; Weinhaupl *et al*., 2000; Shirima *et al*., 2003).

Besides mastitis render milk unsuitable for human consumption, it provides a mechanism for the spread of many diseases to humans (Radostitis *et al*., 1994). Most important human disease causing organisms that can be found in milk are Mycobacterium bovis and tuberculosis, Brucella species, Salmonella species, *E.coli*, *Staphylococcus aureus*, *Streptococcus progeny* and *Corynbacterium haemolyticum* (Table 3). Milk and milk products have, therefore, pose a risk to consumers if it is contaminated by any pathogens and subjected to temperature abuse, where these organisms can multiply to high counts and may produce toxins (Singh, 1994).

Table 3 Important Human Disease Causing Organisms that can be Found in Milk

No.	Name of bacteria	Their effect and disease condition in humans
1	Mycobacterium bovis and tuberculosis	Tuberculosis
2	Brucella species	Undulant or Malta or Mediterranean fever
3	Salmonella species	Salmonellosis
4	*E.coli*	Toxigenic micro organisms
5	*Staphylococcus aureus*	Intoxication
6	*Streptococcus progeny*	Otitis media, septicemia
7	*Corynbacteriumhaemolyticum*	Pharyngitis, cervical adenitis

Source (Singh, 1994).

Milk contains an unacceptable high level of antibiotic residues, so causes problems to consumers of such milk and its products. Drug residues in milk apart from other hazardous effects it also affects negatively the health of the consumer of milk with high level of antibiotic residues. These effects include allergic reactions and bacterial resistance in the body of humans (Muhammad, 2014).

2.8. Status and Significance of Mastitis in Ethiopia

Ethiopia is believed to have the largest livestock population in Africa. An estimate indicates that the country is a home for 59.5 million cattle (CSA, 2017) with the largest member of cows (Almaw *et al*., 2008).Ethiopia is best for dairy development due to its cattle population and favorable climate conditions. The contributions of dairy sector for smallholder poverty alleviation are considerable to be high. However, many factors are constrained by disease like mastitis (Getahun *et al*., 2008) especially subclinical one. Smallholder farmers in Ethiopia are not well informed about the invisible loss from sub clinical mastitis. This is for the reason that dairying is handled as a sideline business among farmers (Abunna *et al*., 2013).Prevalence of subclinical mastitis in Ethiopia and other African regions may impose substantial costs due to indirect losses (Petrovski *et al*., 2006; Halasa *et al*., 2007). Over the last several years, a number of studies are available that describe the prevalence of bovine mastitis in different parts of the country (Table 4). But a number of epidemiological studies carried out in Ethiopia showed that mastitis is a serious problem.

Table 4.Prevalence of Mastitis in Different Area of Ethiopia

Study area	Sample size	Diagnostic Method	Positive animal %			Source
			Clinical	Subclinical	Overall	
In and Around Wolayta Sodo SNNPR, Ethiopia	349	CMT & milk culture	2.6%	26.9%	29.5%	Yohannis & Molla, 2013
Holleta Agricultural Research Center, Ethiopia	90	CMT	7.8%	73.3%	81.1%	Duguma *et al*., 2014
Sidama Zone SNNPR, Ethiopia	96	CMT & milk culture	2.08 %	42.7%	42.71%	Tekle & Berihe, 2016.
Diredawa City, Eastern Ethiopia	334	CMT& milk culture	15.27%	84.73%	39.2%	Kedir*et al*., 2016
Addis Ababa, Central Ethiopia	444	CMT	21.2%	46.8%	68.0%	Tilahun & Aylate, 2015
Horoguduru Wollega zone, western Ethiopia	154	CMT	10.39%	36.36%	46.75%	Beyene & Tolosa , 2017
Ambo West Shewa zone, Oromia, Ethiopia	302	CMT	9.9%	31.8%	41.7%	Sarba & Tola, 2017
Holeta Agricultural Research Center, Ethiopia	186	CMT& milk culture	5.37%	65.06%	70.43%	Dereje *et al*., 2018
Tigray region, Wukro Ethiopia	360	CMT	9.4%	26.7%	36.1	Fentahune *et al*,2018
Benchimaji, SNNPR, Ethiopia	384	CMT& milk culture	30.17%	69.83%	30.21%	Gemechu *et al*.,2018
Hwasa, SNNPR Ethiopia	529	CMT& milk culture	3.4%	59.2%	62.6%	Abebe *et al*.,2016
Holeta Agricultural Research Center, Ethiopia	107	CMT CMT& milk culture	22.4%	48.6%	71%	Mekebib *et al*.,2010
Borena, Oromia Ethiopia	460	CMT& milk culture	21.1	38	59.1	Adane *et al.*,2012

The economic loss from mastitis in the urban and peri urban area of Addis Ababa is U$ 58 and 78.65 per cow and per lactation, respectively (Mungube *et al*., 2005). Losses due to mastitis are commonly derived from sub-clinical and clinical mastitis and their effects are reflected on milk production, composition and quality. The magnitude of these changes in individual cows varies with severity and duration of infection and the causative microorganism that cause mastitis (Radostits *et al*., 2000).

Reported a substantial economic loss in Ethiopian highland crossbred dairy cows is due to subclinical mastitis. However, still there is a gap in Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects (Megersa *et al.,* 2010; Mekibib *et al.,* 2010).

2.9. Diagnosis of Bovine Mastitis

Diagnosis of clinical mastitis is by physical examination including swollen quarters/udder and poor milk quality, can be detected by farmers (Radostits *et al*., 2007; Mahmmod, 2013). The most frequently used diagnostic methods for sub clinical mastitis detection are California mastitis test, somatic cell counting (SCC) and bacteriological culturing of milk (Zadoks and Schukken, 2006).

Physical Examination: This involves close clinical examination of the mammary gland for any signs of inflammation, milk for its color, viscosity. This can be done through visual examination of the milk and mammary gland and or palpation of the mammary gland (Radostits, 2001).

California Mastitis Test (CMT): The California Mastitis Test (CMT) is useful technique for detecting subclinical mastitis on farm, providing an immediate result and for selection of the samples for the bacterial culturing from the cows under (Radostits *et al*., 1994). It conducts in each quarter milk sample immediately after collection. A drop of milk, nearly 2 ml from each quarter placed in each of the four wells of the CMT paddle and an equal amount of the CMT reagent applied to each cup. Gentle circular movements apply to the

mixture, in a horizontal plane for seconds. The obtained reaction result classify as Negative, Trace, 1, 2 and 3 (NMC, 1999).

Somatic Cell Count (SCC): Somatic Cell is normal Constituent of milk consists of different cell types, including neutrophils, macrophages, lymphocytes and some epithelial cells (Sordillo *et al.*, 1997). The somatic cell count (SCC) is the number of cells present in milk. Determination of SCC is widely used to monitor udder health. The increase in SCC during mastitis is part of the immune defense system of the cow. SCC of milk in healthy mammary glandis lower than 1×10^5cells/ml, while bacterial infection can cause it to increase to above 1×10^6cells/ml (Bytyqi *et al.*, 2010).

Measurement of pH: Normal milk has pH between 6.5 and 6.7. When infection is present that it tends toward alkalinity with the use of reagent sodium hydroxide (Chipper, 2000).

Bacteriological Diagnosis: The laboratory procedure of inoculating standard volume of hygienically collected milk on agar culture medium has been the standard diagnostic method for bovine mastitis. The resulting bacterial growth is observed, quantified & tested (Radostits *et al.*, 2007).Bacterial isolates was identified based on colony morphology, pigmentation, Gram stain and conventional biochemical tests. For Gram – positive cocci, catalase tests with hydrogen peroxide (3%) used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Morphology, haemolysis patterns, coagulase and polymyxin susceptibility test was used to distinguish *Staphylococcus aureus*from non-*aureus Staphylococci*. Gram-negative bacteria identified by using colony morphology, oxidase test and lactose fermentation on Macconkey agar (NMC, 1999).

2.10. Treatment of Bovine Mastitis

The specific Anti-microbial therapy during dry period is the best method to eliminate existing infection. Mastitis treatments can be administered at two different lactation cycle stages in lactating cow therapy administered to cows while they are in milk and dry cow therapy administered when the cow is dried off. Mastitis treatment can be administered

by different routes by Intramammary treatment infused into the udder through the teat canal and Parenteral treatment given by injection (Blowey and Edmondson, 2010).

2.11. Control and Prevention

Control of mastitis requires understanding of its causes and management techniques which limit the spread of infection. The principle of mastitis control is that the disease is controlled by either decreasing the exposure of the teat to potential pathogens or by increasing resistance of dairy animals to infection. The key elements in the control of mastitis include: sound husbandry practices fly control, long-acting intramammary antibiotics and sanitation, post-milking teat dipping, treatment of mastitis during non-lactating period, and culling of chronically infected animals (Blowey and Edmondson, 2010).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conduct in Durame town and Kedida Gamela district of Kembata Tembaro zone. Kembata Tembaro is one of the zones of Southern Nation, Nationality and Peoples Region (SNNPR) in Ethiopia. The zone is bordered on the south by Wolayita, on the southwest by Dawro, on the northwest by Hadiya, on the east by the Alaba special woreda, and on the southeast by Hadiya Zone. The administrative center is Durame town. The zone is divided into three agro- ecological zones, such as highland (dega), mid highland (woina dega) and lowland (kolla).

Durame is a town and the administrative center of the Kembata Tembaro Zone. The town has a latitude and longitude of 7°14' N 37° 53'E with an elevation of 2101 meters above sea level. It is located at a distance of 119 km away from capital city of the region, Hawassa and 350 Km away from capital city of Ethiopia, Addis Ababa by Shashemene road. It is surrounded by Kedida Gamela district. The cattle population is 13,809 (KembataTembaro zone Socio-Economic and Geo-Spatial Annual Statistics 2018).

Kedida Gamela is one of the Part of Kembata Tembaro Zone (KT), bordered on the east and south by Hadiya Zone, on the west by Kacha Bira, on the northwest by Angacha, on the north by Damboya, and on the northeast by the Bilate River which separates it from Alaba. It lies 7°14' 60.00" N latitude and longitude 37° 54' 59.99" E with the altitude ranges from 1700 to 3028 meters above sea level. Its area is divided into 7% highland (*Dega*) and 93% *Weyna Dega* (sub-tropical climate).Livelihood of farmers in the area is depending on livestock rearing and crop production. The cattle population is 144,383 (KembataTembaro zone Socio-Economic and Geo-Spatial Annual Statistics 2017).

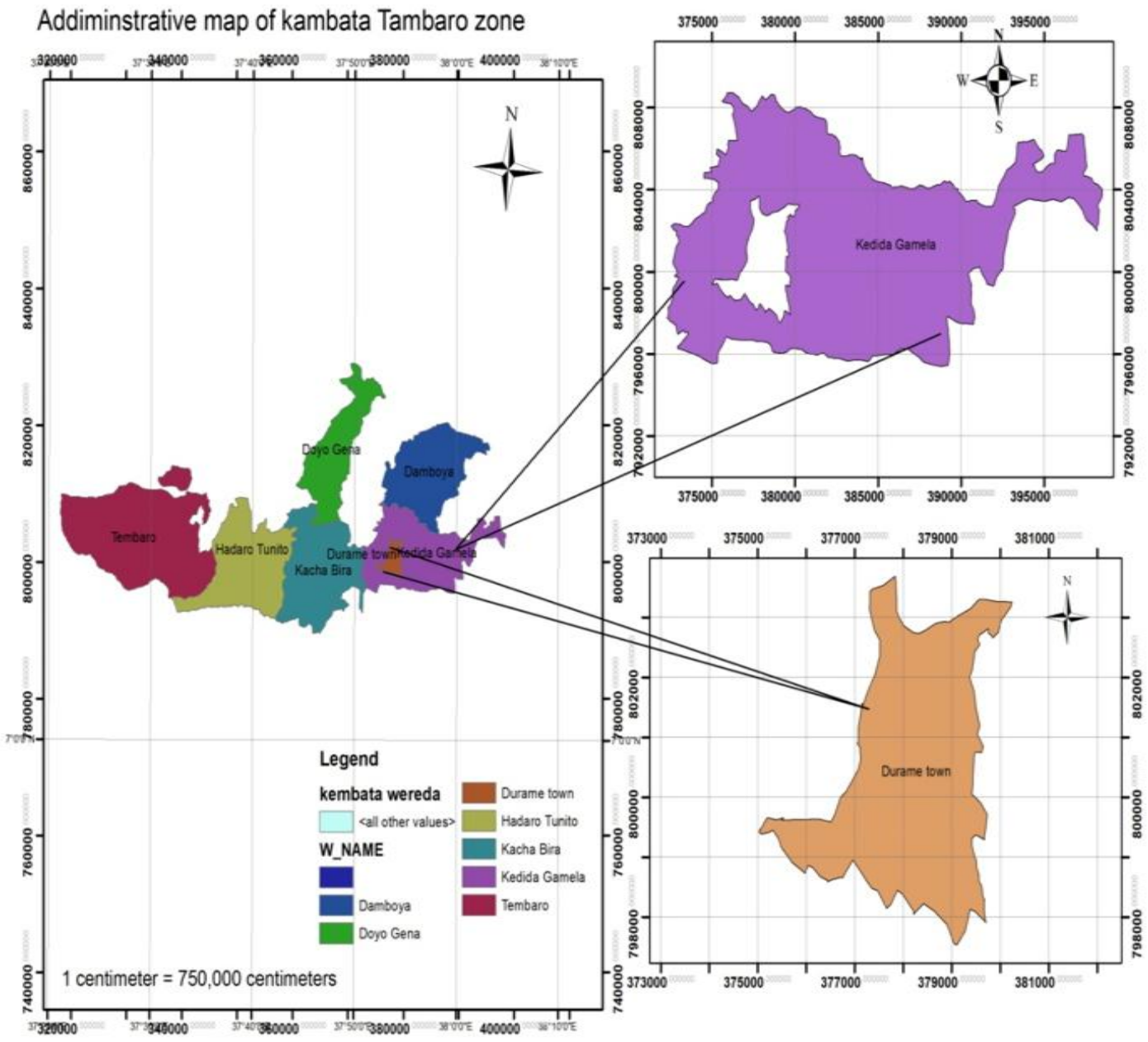

Figure 1.Adminstrative Map of Kembata Tembaro zone indicates study area

Source: Kembata Tembaro zone Socio-Economic and Geo Spatial Annual Statistics, 2018

3.2. Study Animal

The study animals were cross- breed and indigenous zebu lactating dairy cows with different age groups, lactation stages and parity.

3.3. Study Design

Cross sectional study design was conducted between January 2019 and September 2019.

3.4. Sample Size Determinations and Sampling Method

The sample size was determined according to the formula given by Thrusfield (2005). Using 95% confidence interval with 5% absolute precision and at 50% expected prevalence. Since no previous study was conducted in the areas. The 50% expected prevalence is used to calculate the sample size.

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

$$\frac{1.96^2 (0.5) (1-0.5)}{(0.05)^2} = 384 \text{ cow}$$

Where: n= is the required sample size

Pexp = the expected prevalence (50%)

d = is the desired absolute precision (0.05)

z = value at 95% (1.96) from normal table

Therefore, the calculated total sample size was 384 lactating cows.

The study areas, KedidaGamela district and Durame town were purposively selected based on the availability of potential lactating dairy cows. A Simple random sampling technique was used to select the Peasant Associations (PAs), households and individual dairy cow. From the total 18 PAs in the Kedida Gamela district, 12 PAs were randomly selected and from the total 3 PAs in Durame town all 3 PA were selected. Proportional allocation was made for each district. From the total of 417 households 125 (111 from Kedida Gamela and 14 from Durame) were selected randomly based on proportional allocation of lactating cow potential of which 81 were small (1-5 cow), 37 medium (6-10cow), and 7 large (≥10 cow) (Mekonnen, 2018).In each district, the respective

sampling frame was collected in collaboration with livestock and fishery department with each household had at least two lactating cow was involved in the study. Based on proportional allocation of lactating cow potential 2442 lactating cow in Kedida Gamela district and 408 lactating cow in Durame town were sampling frame. To identify lactating individual cow, each animal from the sampling frame list picked randomly by the simple random sampling method using computer generated random numbers and then 329 lactating cows from the Kedida Gamela district and 55 lactating cows from Durame town were selected.

3.5. Study Methodology

Physical examination of udder: Any physical abnormalities for clinical mastitis such as swelling of the udder, presence of lesions or anatomical malformations was recorded and the milk was examined for its color, odor, consistency and other abnormalities.

Milk Samples Collection, Transportation and Storage: Milk samples were collected aseptically from all lactating cows according to the standard procedure of National Mastitis council guidelines (NMC, 1999). The quarter was palpated and first streams of milk were inspected to detect abnormalities. After collection, milk samples were kept in a cool box during transportation to the laboratory. Specified samples processing was performed at Wolayta Sodo regional veterinary laboratory.

California Mastitis Test (CMT): The California Mastitis Test (CMT) was carried out in the field as a screening test for subclinical mastitis and selection of the samples for the bacterial culturing from the cows under study (Radostits *et al*., 1994). A drop of milk, nearly 2 ml from each quarter was placed in each of the four walls of the CMT paddle and an equal amount of the CMT reagent applied to each cup. A gentle circular movement was applied to the mixture, in a horizontal plane for seconds. The reaction obtained is the results was classify as 0 (negative), Trace, 1, 2, 3 (NMC, 1999). Cows were considered positive when at least one quarter turns out to be positive for CMT (Trace, 1, 2, and 3 scores).

Microbiological procedures: Samples from CMT positive and clinical mastitis cows were analyzed microbiologically to identify the causative organism involved in the disease. Bacteriological culture was performed according to NMC guidelines (1999). Loop full milk sample was streaked on blood agar and incubate aerobically at 37°C for 24hrs or 48hrs. A mammary quarter was considered culture-positive when the growth of at least one colony was detected on the streaks. For the primary isolation organisms were identified based on colony morphology, size, shape, color, pigmentation, gram stain, and hemolysis. To detect gram positive and negative bacteria colony was, subculture on MacConkey agar and nutrient agar. To get pure culture, colonies were sub cultured on Mannitol salt agar and Edward's medium. Biochemical test such as catalase test, KOH, Maltose test, Coagulase test, CAMP test, TSI, MBE and Indol test were used for species identification. For gram positive cocci, catalase tests with hydrogen peroxide (3%) were used to differentiate between catalase positive staphylococci and catalase negative cocci. Morphology, haemolysis patterns, coagulase test and polymyxin susceptibility test were used to distinguish *Staphylococcus aureus* from non*aureus Staphylococci.* Gram negative bacteria were identified by using colony morphology, KOH and lactose fermentation on MacConkey agar and TSI (Triple Sugar Iron Test).

Questionnaire: A semi-structured questionnaire was used to collect data regarding the different potential risk factors like breed, age, lactation stage, parity, housing condition, floor type, barn hygiene, udder wash, milking, feeding, towel using, milking frequency and hand washing. Animal data were collected by interviewing responsible personnel. Other farm information was collected by observation to farms, physically to inspect for cleanness, handling, milking procedures and other factors associated with mastitis.

Antimicrobial sensitivity testing: Antibiotic susceptibility screening was done as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique and antibiotic discs and Mueller- Hinton Agar was used. In the preparation of the inoculation cultured broth the McFarland standard was used to cross-checked the turbidity. The antibiotics discs were kept at room temperature for 1 hour before use. A loop full of colony from the growth of isolates was

transferred to the nutrient broth in tubes and incubated at 37°C for 5 h. Mueller-Hinton agar which was used as plating medium was inoculated with broth (bacterial suspension) by using a cotton swab. Then antibiotic discs were applied and pressed onto the plate with forceps. Plates were incubated at 37°C for 18 h. The diameters of zones of growth inhibition were measured in millimeter and interpreted as sensitive, intermediate and resistant to different antibiotics (Quinn *et al*., 1999) and use the National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone. The drugs used were Tetracyclines, Ampicillin, Penicillin, Amoxacillin, Gentamycin and Polymixin.

Estimation of Milk Yield Loss Due to Sub Clinical Mastitis:

A split-udder trial was carried out to collect data on quarter milk loss. Lactating dairy cows were selected from three large farms. All cows were almost under similar management and housing conditions. Also, the cows were with varying lactation stages and parities to reduce the effect of variations within cows to the minimum. Another inclusion criterion in the split-udder is based on cow had to have at least one healthy quarter (Mungube *et al.,* 2005). Each quarter of the study cows were examined using the California Mastitis Test and assumed to be constant throughout the 8-day study period. Milk yield losses in CMT positive quarters estimated by comparing the production of quarters with CMT score 0. Quarters with a CMT result score of negative or zero, trace, 1, 2 and 3 milk yields were compared. Each cow was milked over 8-day period and collects milk from each quarter measured separately (Mungube *et al*. 2005 and NMC, 1999). After the data collected only 18 dairy lactating cross breed cow were obtained based on the above criteria, thus, 23 quarters had a CMT score of "0" while 20, 20, and 9 had 1, 2, and 3, respectively result was obtained. Quarters with CMT trace and 1 were combined, as the difference in the milk yield between the two CMT scores was considered negligible (Mungube *et al.,* 2005). Although cows were milked twice daily, only the late-afternoon milking was used for this study.

To estimate the quarter milk loss due to sub-clinical mastitis, the number of different CMT scores of the positive quarters was multiplied by the corresponding milk production

using the formula given below. The data obtained from Mekonnen *et al*., (1985) who reported that a healthy crossbred dairy cow in Ethiopia yielded a mean of 8.8 kg of milk per day considered as average milk yield and a total mean of 2,896 kg over a 328-day lactation period. Current milk prices used in the calculations was obtained from the trade and industry office.

$$MLy = \frac{(CMT0_y x\ ML_{CMT0}) + (CMT1_y x\ ML_{CMT1}) + (CMT2_y\ x\ ML_{CMT2}) + (CMT3_y\ x\ ML_{CMT3})}{CMT0_y + CMT1_y + CMT2_y + CMT3_y}$$

where ML_y, milk yield loss per quarter in the respective farm level; CMT0 y, 1 y, 2 y, 3 y, number of quarters with respective CMT scores in the farms; $ML_{CMT0,1,2,3}$, production losses determined in the split-udder trial.

The losses are expressed as losses per cow per lactation. In this study, economic loss estimates were only of financial losses derived from milk lost in the infected quarters. Although the health status of an individual quarter could not be taken as a constant throughout an entire lactation, it was assumed that the overall health status of the study population was more or less constant, i.e., that forever case cured there was a new case in another quarter.

3.6. Data Management and Statistical Analysis

Data of physical examination of udder, CMT scores, culture conditions, results of the questionnaire, data on antimicrobial susceptibility and data from the split-udder investigation was recorded into Microsoft excel spreadsheet. It was summarized and analyzed using statistical package for social sciences (SPSS) software (version 23).First the association between the dependent variable, cow mastitis status (0 = negative and 1 = positive) and categorical independent variables was cow and herd related risk factors assessed using univariable logistic regression analyses. The degree and measure level of associations between dependent and independent variables was tested by multivariable logistic regression models and odds ratio (OR).

The independent variables evaluated were breed, parity, stage of lactation, age , floor type, barn cleaning frequency, udder wash material, milking frequency, hand wash

between milking, mastitis cow milking priority, feeding practice, towel using, drainage, ventilation and bedding. All variables with p-value < 0.25 in the initial univariable analysis were checked for multicollinearity using Kruskal gamma statistics and those variables whose gamma value ranged between −0.6 and +0.6 were considered in a multivariable logistic regression analysis to construct the likely model (Dohoo,2009) . The final model was built in backward selection method. In this analysis statistical significance was set at $p < 0.05$.Those factors significant in univariable logistic regression and insignificant in multivariable logistic regression were considered as confounding. Whereas, those factors significant at all level of analysis were considered as risk factors for the occurrence of mastitis in the study area. Chi-square tests were used to compare different means of economic losses due to SCM and drug sensitivity test.

4. RESULTS

4.1. Prevalence of Mastitis

Out of a total of 384 lactating cows examined 220 (57.3%) were found to be positive for mastitis, of which 8 (2.1%) clinical and 212 (55.2%) were subclinical cases, of the study animals, 15 Cows had 15 (0.98%) blind quarters, of which, 4 (1.82%) in Durame town and 11 (0.84%) in Kedida Gamela district. All fifteen quarters were with subclinical mastitis cow. From the total positive cows the prevalence of mastitis recorded in Kedida Gamela district was 177 (53.8%) of which 6 (1.8%) clinical and 171 (52%) subclinical while 43 (78.2%) 2 (3.6%) clinical and 41 (74.6%) subclinical in Durame town. The study result indicated that there was a prevalence difference between districts (Table 5). Prevalence of mastitis was higher in the Durame town than the Kedida Gamela districts. From a total of 1536 quarters examined 15 quarters were found nonfunctional and blind quarters were observed in 15 cows (3.9%) of which 4 (1%) in Durame town and 11 (2.9%) cow in Kedida Gamela district.

Table 5.Prevalence of Mastitis at Cow and Quarter Level in Durame Town and Kedida Gamela District

Forms of mastitis at cow & quarter level	Prevalence		
	Durame Town (%) (n= 55)	Kedida Gamela district (%) (n= 329)	Total (%)
Total positive cow	43 (78.2)	177 (53.8)	220 (57.3)
Sub clinical	41 (74.6)	171 (52)	212 (55.2)
Clinical	2 (3.6)	6 (1.8)	8 (2.1)
Total number of quarter	220	1316	1536
Mastitis Positive quarter	75 (34.1)	328 (24.9)	403 (26.2)
Number of blind quarter	4 (1.82)	11 (0.84)	15 (0.98)
Total number of affected quarter	79 (35.9)	339 (25.8)	418 (27.2)
Number of healthy quarters	141 (64.1)	977 (74.2)	1118 (72.8)

All quarters of 384 lactating cows (1536) were checked for the presence of gross abnormalities. Among these, 383 (24.9%) were found to be positive for sub clinical mastitis (considering CMT T, 1, 2, 3 as positive) and 20 (1.3%) with clinical mastitis and 15 (0.98%) teats were found to be blind. Total quarters found to be unhealthy were 418 (27.2%) (Table6). The infection for the right front quarter is found higher for intra-mammary infection (31.3%) followed by the right hind (26.8%), left rear (25.5%) and left front (25.3%)

Table 6.Prevalence of Mastitis at Quarter Level

Quarter	Total	Prevalence			Total
		Blind	Clinical mastitis (%)	Sub clinical (%)	Prevalence (%)
Right front	384	4 (1)	5 (1.3)	111(28.9)	120 (31.3)
Right hind	384	2 (0.5)	6 (1.6)	95 (24.7)	103 (26.8)
Left front	384	6 (1.6)	4 (1.04)	87 (22.7)	97 (25.3)
Left hind	384	3 (0.8)	5 (1.3)	90 (23.4)	98 (25.5)
Total	1536	15 (0.98)	20 (1.3)	383 (24.9)	418 (27.2)

4.2. Epidemiological Risk Factors in Relation to Bovine Mastitis

Prevalence of mastitis to cow and herd related risk factors was determined. From the ten (10) potential factors entered into multivariable logistic regression analysis, eight (8) factors (breed, parity, stage of lactation, house floor, barn cleaning frequency, mastitis cow milking priority, feeding practice and bedding) were statistically significant ($p<0.05$) (Table 9). Those factors significant in univariable logistic regression and insignificant in multivariable logistic regression were detected as confounding between age and Milking frequency. On the other hand, udder wash material, Hand wash between milking, Towel using, Drainage and ventilation did not have significant effect ($p > 0.05$) on the occurrence of mastitis (Table 8).

Table 7. Univariate Logistic Regression Analysis of the Association of Bovine Mastitis in Relation to Cow and Herd Level risk factor

Variable	Category	Total number	Positive	Prevalence (%)	P-value	EXP(B)	95% C.I. for EXP(B)
Breed	Cross	349	209	59.9	0.04	2.517	1.042 – 6.079
	Local	35	11	31.4		Ref	
Age	2.5 – 6 year	107	53	49.5		Ref	
	6 – 9.5 year	206	111	53.9	0.525	1.245	0.634 – 2.444
	> 9.5 year	71	56	78.9	0.052	2.640	0.992 – 7.026
Stage of lactation	< 90 day	195	150	76.9	0.000	5.334	2.328 – 12.221
	90 – 180 day	134	44	32.8	0.125	0.533	0.239 – 1.190
	>180 day	55	26	47.3		Ref	
Parity	1– 3 calves	194	95	49		Ref	
	3 – 6 calves	85	52	61.2	0.174	1.641	0.803 – 3.354
	> 6 calves	105	73	69.5	0.060	1.985	0.972 – 4.053
Ventilation	Yes	254	129	50.8		Ref	
	No	130	91	70	0.764	1.177	0.406 – 3.414
Bedding	Yes	294	146	49.7		Ref	
	No	90	74	82.2	0.004	3.682	1.534 – 8.834
Drainage	Yes	255	124	48.6		Ref	
	No	129	96	74.4	0.253	1.844	0.646 – 5.266
Floor	Concrete	143	73	51		Ref	
	None concrete	241	147	61	0.027	2.229	1.097 – 4.532
Barn cleaning	1 x per day	38	27	71.1	0.099	2.429	0.847 – 6.966
	2 x per day	145	101	69.7	0.007	2.635	1.300 – 5.341
	3 x per day	201	92	45.8		Ref	
Towel using	Use share	298	174	58.4	0.371	1.449	0.643 – 3.264
	Not use	86	46	53.5		Ref	
Udder wash	With cold water	146	93	63.7	0.403	1.430	0.619 – 3.306
	With warm water	238	127	53.4		Ref	
Milking frequency	2 x per day	124	72	58.1	0.104	1.736	0.892 – 3.377
	3 x per day	260	148	56.9		Ref	
Hand wash between milking	Yes	188	102	54.3		Ref	

	No	196	118	60.2	0.298	1.365	0.760 – 2.451
Mastitic cow milked as last	Yes	36	12	33.3		Ref	
	No	348	208	59.77	0.000	8.671	2.676– 28.095
Feeding practice	After milking	285	140	49.1		Ref	
	Before milking	99	80	80.8	0.000	5.319	2.365 – 11.963

Cow-level risk factors: Breed, parity and stage of lactation had a significant ($p<0.05$) effect on the prevalence of mastitis. Higher prevalence of mastitis (59.9%) was observed in cross breeds as compared to local breeds (31.4%). The likelihood of mastitis was 2.491 times higher in the cross breed than local zebu (95% CI 1.050– 5.908, P = 0.038). With respect to parity, 2.624 times higher in cows having six or more calving compared with cows having 1 – 3 calving (95% CI 1.386 – 4.967, P = 0.003). Cows in the early lactation (< 90 day) were significantly more likely to have mastitis than cows in the late lactation stage (> 180 day) and no significant difference was appreciated between early and mid (90 – 180 day) lactation stages (Table 8 and 9).

Herd Related Risk Factors: Analysis of 11 (eleven) herd related risk factors were performed. From which five (5) risk factors such as bedding material, mastitic cow not milked at last, cow feeding before milking, house floor and barn cleaning were more likely associated with the udder infection. Nevertheless, there was no statistically significant ($p>0.05$) difference in between share towel using and no towel using, milking frequency, udder wash material, Hand wash between milking, Drainage and ventilation. Cows in herds without bedding material were more likely to have mastitis than those cows in herds with bedding material. Cows in herds with concrete floor was less likely to have mastitis than those cows in herds with none concrete floor. Cows in herds that mastitic cows did not milk last were more likely to have mastitis than those cows in herds that milking mastitic cow at last (95% CI 1.823 – 13.476, P = 0.002). Herds feeding their cows before milking were more likely to have mastitis than herds feeding their cows after milking (Table 9).

Table 8. Multivariable Logistic Regression Analysis of the Association of Risk Factors

Variable	Category	B	Z/Wald/	OR	95% C.I. for EXP(B)	P > /Z/
Breed	Cross breed	0.912	4.286	2.491	1.050– 5.908	0.038
	Local			Ref		
Stage of lactation	< 90 day	1.946	22.389	6.999	3.126 – 15.671	0.000
	90 – 180 day	-.339	0.764	0.713	0.334 – 1.523	0.382
	> 180 day			Ref		
Mastitic cow	Yes			Ref		
milked as last	No	1.601	9.838	4.956	1.823 – 13.476	0.002
Parity	1 – 3 calves			Ref		
	3 – 6 calves	0.587	2.927	1.798	0.918 – 3.522	0.087
	> 6 calves	0.965	8.776	2.624	1.386 – 4.967	0.003
Feeding practice	After milking			Ref		
	Before milking	1.635	20.441	5.128	2.524 – 10.415	0.000
House floor	Concrete			Ref		
	None concrete	1.192	13.704	3.293	1.752 – 6.189	0.000
Bedding	Yes			Ref		
	No	1.590	16.514	4.904	2.278 – 10.558	0.000
Barn cleaning	1 x per day	1.050	5.045	2.858	1.143 – 7.146	0.025
	2 x per day	1.201	13.995	3.324	1.772 – 6.237	0.000
	3 x per day			Ref		

4.3. Bacteriological Findings

A total of 403 quarters milk sample from clinical and subclinical mastitis positive were collected for microbiological analysis. Out of 403 samples cultured 397 (25.8%) quarters were yielded growth on culture media. Six samples from subclinical mastitis are not shown growth while all samples from (20 quarters) clinical had positive on culture media. The bacteriological analysis showed that 397 bacteria were isolated and the predominant species were CNS 35.5% followed by *Staphylococcus aureus* 30.5%, *Streptococcus agalactia* 15%, *Ecoli* 11.6%, *Staphylococcus intermidus* 1.8%,

streptococcus uberis 4.3% and mixed species 1.3%. The isolated bacteria from clinical and sub clinical mastitis were indicated on Table 7.

Table 9.Prevalence of Different Bacterial Isolates from Milk Sample

No.	Bacteria Species	No. of Isolates		Total (%)
		Sub clinical mastitis	Clinical mastitis	
1	*Staphylococcus aureus*	116	5	121 (30.5%)
2	*CNS*	134	7	141(35.5%)
3	*Staphylococcus intermidius*	6	1	7(1.8%)
4	*Streptococcus agalactiae*	57	3	60(15%)
5	*Streptococcus uberis*	15	2	1717(4.3%)
6	*E. coli*	44	2	46(11.6%)
7	mixed species	5	0	5(1.3%)

4.4. Antimicrobial Sensitivity Test

Descriptive statistics were used to summarize the data. From the total 397 culture positive (20 from clinical and 377 from sub clinical mastitis), 181 isolates were tested for their *in vitro* antimicrobial sensitivity tests (Table10). The isolates effectiveness was recorded, the antibiotic susceptibility test revealed that highest numbers of staphylococcus aureus were susceptible to Gentamycin95% and not susceptible to Polymixyn and Ampicillin and highly resistance to Polymixyn 96.7% followed by Ampicillin 83.3%.The highest numbers of coagulase negative staphylococcus were susceptible to Tetracycline 77.4 % and Gentamycin 69.8%. The antibiotic susceptibility test indicated that all the number of *staphylococcus intermidius* were susceptible to Gentamycin 100% followed by Amoxicillin 75%.The antibiotic susceptibility test suggested that highest number of *Streptococcus* agalactiae were susceptible to Gentamycin85.7% and no susceptible to Ampicillin and pencellin. In antibiotic susceptibility test *Streptococcus uberis* showed highest susceptibility to Gentamycin 100% and Tetracycline 87.5%.The antibiotic susceptibility tests showed that all tested *E.coli* were susceptible toGentamycin100%and highest numbers of *E.coli* were susceptible to Amoxicillin 85.7% and Polymixyn 81 % and highly resistance to Penicillin 90.5 % followed by Tetracycline85.7% and Ampicillin 71.4% (Table 10).

Table 10. Number of Tested Microorganism for Antibiotic Sensitivity Test and Its Effectiveness

Result to Application of Antimicrobial Disk (%)																		
Isolated species	N	Gentamycin			Amoxicillin			Tetracycline			Ampicillin			Penicillin			Polymixyn		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus	60	57 (95)	2 (3.3)	1 (1.7)	4 (6.7)	21(35)	35(58.3)	19(31.7)	31(51.7)	10(16.7)	0	10(16.7)	50(83.3)	4(6.7)	26(43.3)	30(50)	0	2(3.3)	58(96.7)
CNS	53	37(69.8)	12 (22.6)	4 (7.5)	11(20.8)	34 (64.2)	8 (15.1)	41(77.4)	10 (18.9)	2 (3.8)	6 (11.3)	22 (41.5)	25 (47.2)	5 (9.4)	18 (34)	29 (54.7)	10(18.9)	14 (26.4)	28 (52.8)
S. intermidius	4	4(100)	0	0	3 (75)	1 (25)	0	2 (50)	2 (50)	0	2 (50)	2 (50)	0	0	2 (50)	2 (50)	0	2 (50)	2 (50)
S. agalactiae	35	30(85.7)	4 (11.4)	1(2.9)	7 (20)	21 (60)	7 (20)	15 (42.9)	11 (31.4)	9 (25.7)	0	21 (60)	14 (40)	0	24 (68.6)	11 (31.4)	5 (14.3)	30 (85.7)	0
S. uberis	8	8(100)	0	0	2 (25)	6 (75)	0	7 (87.5)	1 (12.5)	0	6 (75)	2 (25)	0	0	1 (12.5)	7 (87.5)	0	2 (25)	6 (75)
E. coli	21	21(100)	0	0	18 (85.7)	3 (14.3)	0	0	3 (14.3)	18 (85.7)	6 (28.6)	0	15 (71.4)	0	2 (9.5)	19 (90.5)	17 (81)	4 (19)	0
Total	181	157 (86.7)	18 (10)	6 (3.3)	45 (24.9)	86 (47.5)	50 (27.6)	84 (46.4)	58 (32)	39 (21.5)	20 (11)	55 (30.4)	104 (57.5)	9 (5)	73 (40.3)	98 (54.1)	32 (17.7)	54 (29.8)	94 (51.9)

Keys: N = Number of tested, S: Susceptible, I = Intermediate, R = Resistance

The effectiveness of antimicrobial antibiotics was presented in Figure 3 and Table 11. Results showed that the majority of isolates were highly sensitive to Gentamycin 178 (98.34%) and resistance for Polymixyn 105 (58.01%) followed by Ampicillin 104 (57.5%).

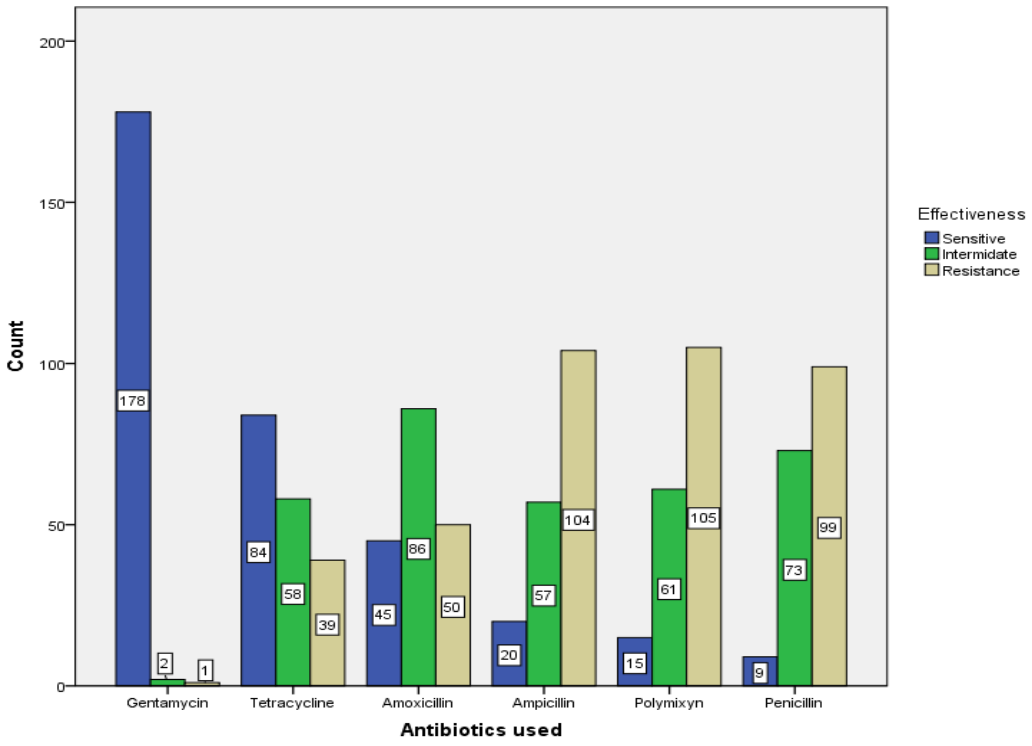

Figure 2: The Effectiveness of Different Antibiotics

Table 11.Antibiotic Sensitivity of Isolates, from Clinical and Sub Clinical Cases of Mastitis

No.	Antibiotic	Tested	S (%)	I (%)	R (%)
1	Gentamycin	181	178 (98.3%)	2 (1.1%)	1(0.6%)
2	Tetracycline	181	84(46.4%)	58 (32%)	39(21.6%)
3	Amoxicillin	181	45(24.9%)	86 (47.5%)	50(27.6%)
4	Ampicillin	181	20(11%)	57 (31.5%)	104(57.5%)
5	Polymixyn	181	15(8.3%)	61 (33.7%)	105(58%)
6	Penicillin	181	9(5%)	73 (40.3%)	99(54.7%)

Keys: S: Susceptible, I = Intermediate, R = Resistance

4.5. Estimation of Economic Loss of Sub Clinical Mastitis in the Study Area

In split-udder trial, the average milk production per healthy quarter (CMT=0) was 0.995 kg (95% CI, 0.74 – 1.25 kg) per milking. The difference in the average milk yield between uninfected quarters (CMT=0), and infected ones with CMT scores of 1, 2, and 3 were 0.062 kg (6.2%), 0.2359 kg (24%), and 0.502 kg (50.5%) respectively. Also, the mean milk production for healthy quarters was significantly different among quarters with different CMT scores while quarters with CMT score 3 had significantly lower yield than those with CMT 1 and 2. An overall milk total production loss per quarter of SCM was estimated at 5.8 % of possible production, and loss in quarters with SCM was 22.7%. The CMT scores from the prevalence study and the associated milk yield losses based on the split-udder investigation are shown in Table 12

Table 12. Quarter CMT Scores and Associated Milk Production Losses at Quarter Level

Level of estimation	CMT 0	CMT1	CMT2	CMT3	Total loss	Loss in quarter with CMT (%)
Loss (%)	0	6.2	24	50.5		
All cow quarter	1118	148	155	80	5.8	22.7
Farm size						
Small (1-5cow)	506	58	61	27	4.9	21.8
Medium (6-10 cow)	425	56	59	30	5.7	22.65
Large (> 10cow)	187	34	35	23	7.9	24.03

In this study the costs of other factors such as decreased milk quality, changed milk composition, labor cost, treatment cost, premature culling and the associated replacement costs contributing to economic losses were not considered because of difficulties in obtaining reliable data. Mungube *et al.,* 2005 reported similar problems in their work on the impact of mastitis in dairy farms.

Milk prices used in the calculations were obtained from the trade and industry office. Farmers were sell milk at price of 15 Eth Birr/kg, 16.70 Eth Birr/kg and 20 Eth Birr/kg

for the community. Economic losses per cow per lactation estimated for different milk prices was 3348.2 Eth Birr(US $112.55), 2795.77 Eth Birr (US $93.98) and 2511.17 Eth Birr (US $84.4).Based on the average price 17.23 Ethiopian birr per kilogram of milk, sub clinical mastitis causes an estimated total loss of 2884.59 Eth Birr (US $96.96) per cow per lactation. This study was higher than that reported by Tesfaye *et al*., 2010 and Mungube *et al.* 2005. The reason for this is increased milk price at the present day. Also, studies revealed that bacterial mastitis is a problem of high producing cows and different mastitis pathogens have shown difference in their pathogenesis, epidemiology and clinical presentations (Sears and Wilson 2003; Grohn *et al.* 2004).Financial losses were higher in the large farms 3928.9 Eth Birr (US$132.1) than medium farms 2834.8 Eth Birr (US$95.3) and small-size farms 2436.9 Eth Birr (US$ 81.9).

5. DISCUSSION

The overall prevalence of mastitis in this study was in line with the findings of Teklesilasie *et al.* (2014) and Abebe *et al.* (2016) who report 52.6% and 62.6% respectively. However, this finding is lower than report of Mekibib *et al*. (2010) who reported71%at Holeta Town, Central Ethiopia. This prevalence is relatively higher than the reports of Yohannis and Molla (2013), 29.5%in and around Wolaita Sodo, Southern Ethiopia and Kedir *et al*. (2016), 39.2% in Dire Dawa City; Eastern Ethiopia.Thedifferences in reports could be due to differences in management systems, breeds of cattle and agro-climatic areas that might contribute to the variability of mastitis prevalence among reports.

The finding of 2.1% of clinical mastitis and 55.2% of subclinical mastitis prevalence in this study is very closely agreed with the findings of Tekle and Berihe (2016) who reported 2.08% and Yohannis and Molla (2013), who reported 2.6%in and around Wolaita Sodo, Southern Ethiopia. However, it was lower than the findings of 14.6% of Teklesilasie *et al*. (2014) and 9.9% Sarba and Tola (2017) in West Shewa Ethiopia. The findings of subclinical mastitis in this research are comparable with the finding reported by Tilahun and Aylate (2015) who reported 46.8%.However, higher than Sarba and Tola (2017) who report of 32.8%. The higher prevalence of subclinical mastitis in the study area could be attributed to the little attention given to it and farmers are not aware about the silent cases of mastitis.

Quarter prevalence of mastitis was 27.2% which was comparable with the finding of Abebe *et al.*(2016)who reported the quarter prevalence of 36%.The present finding was higher than reports made by Yohannis and Molla (2013) who reported 17.9%in and around Wolaita Sodo, Southern Ethiopia. Comparisons of the infection with regard to positions of quarters indicated that the highest infection were in the right front quarters (31.3%) and this finding is in line with the finding of Sarba and Tola (2017).This is explained by the fact that the right quarter might also be the first and ease to be grasped by milkers as routine milking procedures (Radostits *et al*., 2007). The right hind quarters were the second with an infection rate of 26.8%, followed by the left hind which is

25.5% and left front. In the case of hind quarters have high chance of getting environmental and fecal contamination (Sori*et al*., 2005).

Cows with increased parity had increased the risk of mastitis. The result showed that the prevalence of mastitis was 61.2% in cows that had a parity number of 3-6 calves, followed by cows having more than 6 calves 69.5%,This finding was comparable with the result of Asmare and Kassa (2017) 69.6 %in Sodo Town and its Surroundings, Southern Ethiopia. This might be due to cows with advanced parity become more productive, so it can be assumed that as the parity of cows advance and the age increases cows become prone to mastitis and increased opportunity of infection with time and the prolonged duration of infection (Radostits *et al*., 2007).

In this study the prevalence was higher in cows in early lactation (76.9%) as compared to in cow in late stages of lactation (47.3%). The mid stage (32.8%) of lactation was lower than both stages. Girma *et al*. (2012); Yohannis and Molla (2013)and Dego and Tareke (2003) were reported higher prevalence of mastitis during early lactation stage than late lactation from different parts of Ethiopia. The early lactation stage infection might be due to the carryover of infection from dry period. Additionally, most new infections occur during the early part of the dry period and in the first two months of lactation (Radostits *et al*., 2007). In addition the occurrence of more cases during earlier lactation stage may be due to birth related influences (Biffa *et al*., 2005) and at late lactation there is decrement of neutrophil concentration when the cows reach to dry off (Quinn *et al*., 2005). About 25% cases of mastitis are expected to occur between 61 and 100 days of calving, which coincides with peak lactation because this relates to the effect of dry period infections, calving, peak yield and the highest production stress on the cow (Blowey and Edmondson, 2010).

In this study the prevalence of mastitis in crossbred cows (59.9%) was higher than local breeds (31.4%) similar to the reports of other studies; Beyene and Tolosa (2017) who reported 58.46 in crossbred cows and 38.2 in local breed and Tekle and Berihe (2016) reported 58.33 in crossbred compared to 33.33 in local in Sidama Ethiopia. This variation of mastitis prevalence in breed level could be that the disease is associated with high

yielding cows of crossbred to low yielding local breed. Higher-yielding cows have been found more susceptible to mastitis may be due to the position of teat and udder and anatomy of teat canal making them prone to injury (Radostits and Blood, 1994; Radostits *et al*., 2007).

In this study, feeding practice of cows was significantly affected the prevalence of mastitis. Feeding cows directly after milking had the prevalence of 49.1% which was lower than prevalence of mastitis (80.8%) in cows that feed before milking. This could be related to reduced lying down after milking while teat is still open. The cows from lying down while their teats ducts remain open for times and thus is associated with cows teat stay pendulous when cow remains standing until the feeding finish (Bartlett *et al.,* 1992). These avoid contact of cow with contaminated floor and prevent environmental pathogens from freely entering through the open teat canal (Bartlett *et al.,* 1992) as the teat canals may remain partially open for 1–2 hour after milking (Idriss *et al.* 2013). The finding was comparable with the finding of Plozza *et al.* (2011) but opposite with the finding of Asmare and Kassa (2017) reported that feeding cows just after milking were unexpectedly found to be highly risky for incidence of mastitis than cows not fed after milking.

Mastitic cow milking practice had significant effect on the mastitis. Farms that did not milk the mastitic cows last were 59.8% prevalent than milking at last. This result was comparable with the finding of Abebe *et al.* (2016) who reported that 67.9% prevalence in cows not milked at last and 54.0% in cows milked at last in Ethiopia. Failure to milk mastitic cows last would favor spread of mastitis pathogens between cows by milker's hands resulting in contagious mastitis (FAO, 2014).

Coagulase-negative staphylococci were the most frequently isolated bacteria in this study 35.5%, followed by *S. aureus* 30.5%. The result was comparable with the finding of Kedir *et al.* (2016)who reported 34.4%and it was higher than Mekonnen (2018) who reported that 32% in North West Ethiopia. C*oagulase negative staphylococcus* is environmental pathogen not as pathogenic as *S. aureus* and infections usually remain sub- acute. However, CNS causes persistent infections that may result in increased milk SCC, udder damage and decreased milk quality and production (Contreras and

Rodríguez, 2011). In this study, the next predominant isolates were *Staphylococcus aureus*. *Staphylococcus aureus* is well adapted to survive in the udder and usually establish mild infection of long duration from it shed thorough milk facilitating transmission to healthy animals mainly during unhygienic milking procedures.

Escherchia coli were 11.6%, this result agrees with the finding of Dereje *et al.* (2018) who reported 16.12% prevalence in Holleta Agricultural Research Center, Central Ethiopia. This result is relatively higher than Girma *et al*. (2012) who reported 9.92%Prevalence *Escherchia coli* in his study in West Harerghe zone, Ethiopia. Poor barn management of dairy cows may also provide the cows with predisposing factors for coliform mastitis (Ward *et al*., 2002; Krause *et al*., 2003).

The isolation of *Streptococcus agalactiae* was the third isolated in this study. It was comparable with the report of Duguma *et al*. (2014) who reported12.2% in his study in Holleta Agricultural Research Center, Central Ethiopia. However, the present finding is higher than the finding of Dereje *et al*. (2018) who reported5.15%in Holleta Agricultural Research Center, Central Ethiopia. *Streptococcus uberis* was isolated at a rate of 4.3% which is comparable withGirma *et al*. (2012) who reported 5.8 % in West Harerghe zone, Doba district, Ethiopiaand higher than Duguma *et al*. (2014) reported 2.8% in his study in Holleta Agricultural Research Center, Central Ethiopia.

For this finding antimicrobial sensitivity test showed that Gentamycin was the first effective antibiotic in the study area. The finding of this study was in agreement with the report of Dereje *et al*. (2018) and Abera *et al*. (2013). All isolated species were highly sensitive to Gentamycin. However, *Staphylococcus aureus* and CNS were resistant to Polymyxin B, Penicillin and Ampicillin. Whereas majority of the isolates were highly resistant to Penicillin and Ampicillin. In this study *E.coli* was sensitive to Polymyxin B.Polymyxin B is useful for the treatment of coliform mastitis, and their potential to inactivate endotoxin (Du Preez, 2000).

The average quarter milk production of the cows used for split udder trial for healthy quarter was 0.995which would amount to 3.98 kg for all health quarter per milking, if similar production is assumed for second milking then a total of 7.96 kg per day would

be attained. This was in agreement with previous studies reported for the Ethiopian highlands by Mungube *et al*. (2005) who report 0.82 kg per quarter per milking. However, this finding is lower than Tesfaye *et al*. (2010) who reported 1.6 kg per quarter per milking. The split udder trial showed that a quarter with sub clinical mastitis lost on average 22.7% of potential milk production. This result was higher than the finding of Mungube *et al.* (2005) who reported 17.2% and lower than Tesfaye *et al*. (2010) who reported 34.5%.Prevalence of SCM at the farm level was higher in large-scale farms than in small scale farms. The reason for this could be poor milking management and farms where a single milkier has been usually assigned to more than ten cows per milking. In this study the costs of other factors such as decreased milk quality, changed milk composition, labor cost, treatment cost, premature culling and the associated replacement costs contributing to economic losses were not considered because of difficulties in obtaining reliable data. Mungube *et al.,* 2005 reported similar problems in their work on the impact of mastitis in dairy farms.

Farmers were selling milk at price of 15 Eth Birr/kg, 16.70 Eth Birr/kg and 20 Eth Birr/kg for the community. Economic losses per cow per lactation estimated for different milk prices was 3348.2 Eth Birr(US $112.55), 2795.77 Eth Birr (US $93.98) and 2511.17 Eth Birr (US $84.4).Based on the average price 17.23 Ethiopian birr per kilogram of milk, sub clinical mastitis causes an estimated total loss of 2884.59 Eth Birr (US $96.96) per cow per lactation. This study was higher than that reported by Tesfaye *et al*., 2010 and Mungube *et al.* 2005. The reason for this is increased milk price at the present day. Also, studies revealed that bacterial mastitis is a problem of high producing cows and different mastitis pathogens have shown difference in their pathogenesis, epidemiology and clinical presentations (Grohn *et al.* 2004; Sears and Wilson 2003).Losses in small-size farms, medium farms and large farms were 2436.9 Eth Birr (US$ 81.9), 2834.8 Eth Birr (US$95.3) and 3928.9 Eth Birr (US$132.1) respectively.

6. CONCLUSION AND RECOMMENDATION

The present study revealed that subclinical bovine mastitis was found to be one of the major diseases of dairy farms in Durame town and Kedida Gamela district. Of the 15 risk factors examined breed, parity, stage of lactation, bedding material, drainage, mastitic cow milking practice, cow feeding before milking, house floor and barn cleaning frequency were significantly associated with mastitis prevalence. The *Coagulase-negative staphylococci*, *staphylococcus aureus*, *S. agalactiae* and *E. coli* bacteria were the most predominant causes of mastitis in the area. The present study showed that all micro-organisms were susceptible to *Gentamycin* antibiotic which can be used for effective treatment of bovine mastitis in the study area. However, high resistance was observed by most of the isolates towards Penicillin and polymixn antibiotic. Sub clinical mastitis had serious economic losses in dairy cows without notice on smallholder dairy farms by silent reducing of milk production. This implies that mastitis has an overlooked impact on dairy development and food security in the area.

Based on the above conclusion the following recommendation is forwarded

- Smallholder farmers were not well informed about the invisible loss from sub clinical mastitis. Therefore, awareness and training programs should be created to the farmers will have positive effects to avoid associated risk factors, knowledge about transmission and occurrence of mastitis spread at farm level.
- This study examined the quantitative aspect of sub clinical mastitis loss only and could not account for the role of pathogenic agents on the milk loss and loss of clinical mastitis. Therefore, further studies are needed to show the pathogenic microorganism difference effect on the milk production loss and economic loss associated with clinical mastitis.
- More epidemiological studies on bovine mastitis are required in order to have strong scientific data on the transmission of disease, other possible risk factors or diagnostic procedures and public health impact of the disease. It improved and can lead to significant increases in milk quality and quantity in the study area.

- The professionals should design for warrens of control methods for highly pathogenic agent and develop guideline for dairy production management in the country.

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8. APPENDICES

Appendix 1 Questionnaire

Semi-structured Questionnaires on Assessment of Bovine Mastitis and Other Problems of Dairy Farms

Explanation:

The objective of this questionnaire is to determine for the risk factor association of bovine mastitis and factors affecting the milk production.

1. Name of the respondent (owners name)…..……sex…….Address/wereda……..Kebele……..Date…
2. Number of herd in the farm……

3. Origin of sample:

3.1 Breed

a) Cross b) Local

4. Physiologic state of the mammary gland

4.1. Lactating ……

4.2. Milk in liter per day ……

4.3. Stage of lactation

a) Less than 90 day

b) 90 – 180 day

c) Greater than 180 day

5. Clinical state of cow

5.1. Clinically mastitis

5.2. Apparently normal

5.3. If you have more than one milking cow, when a cow has mastitis, do you milk it last?

a) Yes at last b) Not at last

6. Parity a) 1-3 calves b) 3-6 calves c) greater than 6 calves

7. Age of cow a) 2.5-6 years b) 6-9.5 years c) greater than 9.5 years

8. Management history

8.1. Feeding practice

a) Feeding before milking b) Feeding after milking

8.2. Housing

8.2.1. Floor of house a) Concrete b) None concrete

8.2.2. Drainage a)With drainageb)Without drainage

8.2.3. Ventilation a)With ventilation b)Without ventilation

8.2.4. Bedding a)with bedding b)Without bedding

8.2.5. Barn cleaning frequency a) per day b) 2 times per day c) >2 times per day

8.3. Milking practice

8.3.1. Are udders and teats washed before milking? a) Yes b) no

8.3.2. Is a towel used for each cow? a) Use share towel b) not use towel

8.3.3. Are hands washed before and between milking? a) Yes b) no

8.3.4. Milking frequency a) twice per day b) three times per day

8.3.5. Udder cleaning materials a) tap water b) warm water

THANK YOU

Appendix 2 The California Mastitis Test (CMT) Procedure

Performed at milking time discard the first squirt of foremilk

Squirt milk from each quarter into a different well on the CMT test tray (approximately 2 ml from each quarter)

Mix each milk sample with an equal volume of reagent (available commercially)

Swirl the mixture in a circular motion with presence of gel or slime being recorded for each quarter vigorously for maximum of 20 seconds and examine the degree of thickening/gelling in each sample (gelling may be more visible if the test tray is tilted)

The obtained reaction result classify as Negative, Trace, 1, 2 and 3

Appendix 3 Blood agar preparation

Ingredients: Trypticase or Tryptic Soy Agar Powder…….40g

Purified (distilled or deionized) water……..1000 ml

Procedures

Mix thoroughly and heat with frequent agitation: boil for 1 minute to completely dissolve the agar.

Dispense in to 200-ml aliquots using 250-ml flasks with vented stoppers or aluminum foil.

Sterilize by autoclaving at 15lb pressure (121c) for 15 minutes.

Place sterile media in water bath set at 45 to 50 c for 1 hour. NOTE: if agar is being prepared for later use. It may be stored at room temperature for up to 5 days; otherwise. It should be refrigerated at approximately 6 c.

After 1 hour, add defibrinated ovine blood to a final concentration of 5% and swirl gently. Mix well and pour in to petriplates using aliquots of 12 to 14 ml for each 100 x 15-mm plate. The yield is approximately 15 to 18 plates/200 ml of sterile media.

Allow agar to solidify and incubate inverted at 37 c for 18 to 24 hours to reduce excess moisture and moisture and to check sterility of the medium.

Store inverted in a refrigerator at approximately 6 c until use

Appendix 4 Nuterent agar preparation

Approximate Formula* Per Liter

Ingredients: Beef Extract..... 3.0 g

Peptone..... 5.0 g

Agar..... 15.0 g

Procedure

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.

4. Test samples of the finished product for performance using stable, typical control cultures.

5. Cool to 45-50°C and pour into Petri dishes. Allow to solidify for at least 30 minutes. Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-24 hours and 42-48 hours, if necessary.

Appendix 5 Mannitol Salt Agar preparation

Approximate Formula* Per Liter

Ingredients: Pancreatic Digest of Casein..... 5.0 g
Peptic Digest of Animal Tissue.....5.0 g
Beef Extract..... 1.0 g
Sodium Chloride..... 75.0 g
D-Mannitol..... 10.0 g
Phenol Red..... 25.0 mg
Agar..... …15.0 g

Procedure

1. Suspend 111g of the powder in 1L of purified water. Mix thoroughly.

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes.

4. Test samples of the finished product for performance using stable, typical control cultures.

Appendix 6 Antimicrobial susceptibility tests Kirby–Bauer disc diffusion method

Kirby–Bauer disc diffusion method is the most common method used routinely for determination of antibiotic sensitivity of bacteria.

Required:

- Sensitivity testing media / Mueller-Hinton agar/
- Anti-microbial discs
- Control strains
- Turbidity standard

Method

Emulsify several colonies of similar appearance of the test organism in a small volume of sterile nutrient both.

Both the test strains and the control strains are tested in separate plates.

The test is performed by inoculating the test organism in a suitable broth solution, followed by incubation at 37°C for 2–4 hours.

Match the turbidity of the subculture against the turbidity standard.

Apply a loopful of the test organism subculture to the sensitivity testing plate using a sterile loop.

Spread the inoculum evenly across the plate using a sterile dry cotton wool swab.

Allow the inocula to dry for a few minutes with the petridish lid in place.

Place the anti microbial discs(obtained commercially) into the test organism in petridish using a sterile forceps or dispenser.

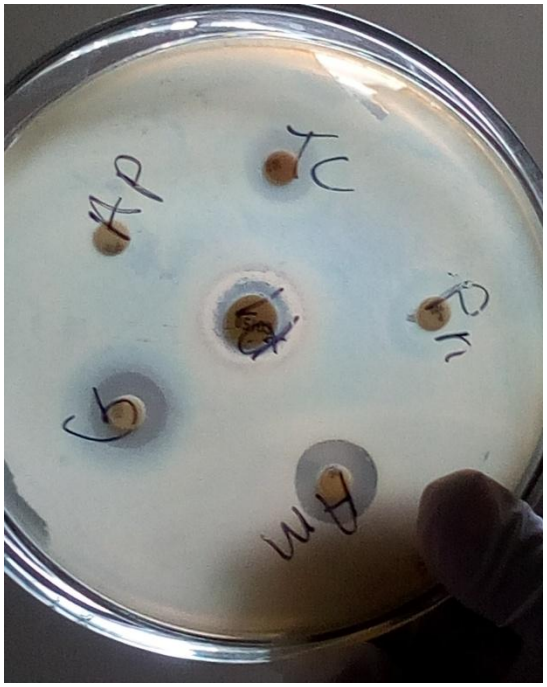
NB: each disc should be pressed down on the medium and should not be moved once in place.

Incubate the plate aerobically at 37°C for 18–24 hours after 30 minutes of applying the discs

Read the tests and interpret as ‘sensitive (S)’, “resistant (R)”or “intermediate (I)” comparing the chart of the sensitivity test**.**

A maximum of six antibiotic discs are tested in a Petri dish of 85 mm size.






Mueller Hinton Agar preparation

Approximate Formula* Per Liter

Beef Extract Powder..... 2.0 g

Acid Digest of Casein..... 17.5 g

Starch.....1.5 g

Agar.....17.0 g

Procedure

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes. Cool medium to 45 50°C and aseptically add 5% sterile defibrinated sheep blood.

4. Pour cooled Mueller Hinton agar into sterile Petri dishes on a level, horizontal surface to give a uniform depth of about 4 mm (60-70 mL of medium for 150 mm plates and 25-30 mL for 100 mm plates) and cool to room temperature.4

5. Check prepared medium to ensure the final pH is 7.3

6. Test samples of the finished product for performance using stable, typical control cultures.

Appendix 7 Coagulase Tube Test

Procedure

Inoculate 0.5 ml Coagulase plasma with a heavy inoculums of staphylococci from a 24-hour plate culture. Use a wire loop or the end of a sterile applicator stick for inoculation.

Incubate tubes at 37 c in a water bath or an incubator for up to 24 hours.

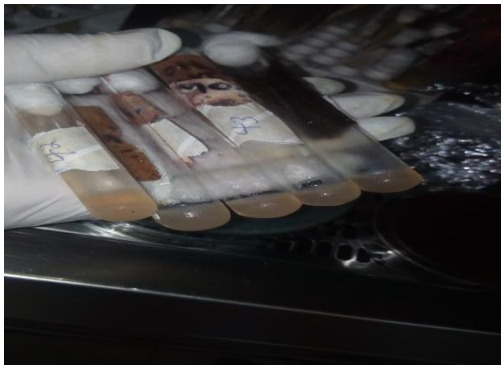
Interpretation positive reaction

Semi- solid to solid gelling evident when tube is tipped. Eg. Staphylococcus aureus

Negative reaction

Liquid state after 24 hours incubation

Eg. Staphylococcus chromogenues



Appendix 8 Catalase Test

Procedures

Put a drop of 3% solution of hydrogen peroxide on a microscope slide.

Emulsify a colony in the peroxide.

Interpretation

Positive reaction bubbles are produced eg : Staphylococci

Negative reaction no reaction eg. Streptococci

Appendix 9 Edwards modified medium

Ingredients: lab-lemco powder ……..10g

Peptone……10g

Esculin…….1g

Sodium chloride…….……..5g

Crystal violet…….0.0013g

Thallous sulfate…….0.33g

Agar ……..……15g

Sterile bovine or ovine blood…….……50 ml

Distilled water…….…..950ml

Procedure

Mix ingredients except sterile blood and heat to dissolve

Sterilize at 15 lb pressure (121 c) for 20 minutes.

Temper at 50 c for 1 hour.

Add sterile blood and swirl gently

Pour 13 to 15 ml per plate.

Store inverted in refrigerator.

Appendix 10 MacConkey agar preparation

Ingredients: MacConkey agar powder……50g

Distilled water……..…….1000ml

Procedures

Mix solution and heat to dissolve

Sterilize at 15 lb pressure (121 c) for 15 minutes.

Temper sterile media at 47 to 50 c for 1 hour,

Pour 13 to 15 ml per plate

Store inverted in a refrigerator.

Appendix 11 Gram stain

Prepare dry smear

Make a slide from a pure culture by mixing a small amount with a small drop of sterile distilled water or sterile bronth. Mark slide with wax pencil to locate smear. Air dry and fix by lightly passing slide through flame, being careful not to burn.

Flamed slide should be able to hold on wrist without feeling too hot.

Staining

Apply crystal violate to smear by flooding slide for 30 to 60 seconds.

Wash off with tap water.

Apply Gram's iodine for 30 to 60 seconds. Drain do not wash

Decolorize by continual gentle rinsing with 95% alcohol just until color is no longer present in run off.

Rinse with tap water.

Apply safranin for approximately 1 minute.

Rinse away stain with tap water.

Blot dry with filter paper and examine.

Appendix 12 KOH test

A simple and effective method for determining the Gram staining reaction of bacteria is the KOH test. The only reagent required is a 3% aqueous solution of potassium hydroxide and the result has correlated closely with Gram staining results.

Procedure

Place a drop of 3% KOH onto a microscope slide.

Transfer one or more like colonies from the surface of solid medium into the KOH solution on the slide

Mix and read within 60 seconds.

Interpretation positive reaction mixture becomes viscous or gels eg. Gram – negative bacteria

Negative reaction mixture remains fluid eg. Gram positive bacteria

Appendix 13 Triple sugar iron agar (TSI)

Principle

Triple sugar iron (TSI) slant agar is used to determine whether an organism ferments glucose, lactose, and sucrose. A TSI slant begins as an orange- or red-colored agar at an alkaline pH. Phenol red is the pH indicator and ferrous sulfate with sodium thiosulfate detects hydrogen sulphide production. If any of the carbohydrates are fermented, an acid pH will result, and either the butt or the slant and butt will turn yellow. In addition to peptone, yeast extract & agar, it contains 3 sugars – Glucose, Lactose, and Sucrose. The Objective to study different properties of a bacterium – sugar fermentation, gas production and H2S production.

Materials: TSI agar, test bacteria, two types of inoculating needles (straight and wire loop)

Procedure

a) Inoculate the bottom of the TSI agar with the straight needles.

b) Using the wire loop streak the slant by stabbing to the bottom and then inoculating the surface of the slant as you withdraw the needle.

c) Cap very loosely and incubate overnight at 37 0C for 24 hours and write your observations after 24 hours.

Result

Yellow – Acid

Pink (red) - Alkaline

Yellow slant – Lactose fermenters.

Pink slant – Non lactose fermenters.

Pink slant / no colour change – Non fermenters

Black colour – H2S production.

Gas bubbles or crack in the medium – gas production.

Lactose Fermentors – E.coli, Klebsiella

Non Lactose Fermentors – Salmonella, Shigella

H2S producers- Proteus

Appendix 14 CAMP test

Procedure

Streak a beta-lysin–producing strain of *aureus* down the center of a sheep blood agar plate.

The streptococcal streak should be 3 to 4 cm long.

Streak test organisms across the plate perpendicular to the *aureus* streak within 2 mm. (Multiple organisms can be tested on a single plate).

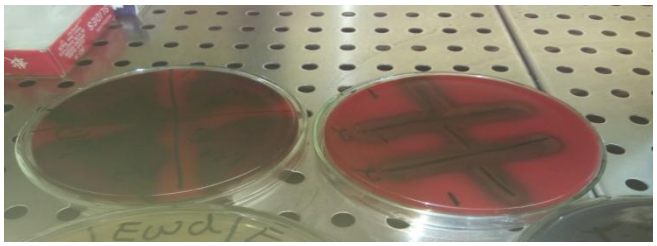
Incubate at 35°-37°C in ambient air for 18-24 hours.

Group B streptococci and a few other beta-streptococci produce an enhancement of the ß-lysin activity of the *aureus* strain.

Result Interpretation of CAMP test

Positive: Enhanced hemolysis is indicated by an arrow head-shaped zone of beta-hemolysis at the junction of the two organisms.

Negative: No enhancement of hemolysis.



Appendix 15 Indole test

Indole test is used to detect the ability of bacteria to decompose amino acid tryptophan to indole, which accumulates in the medium. Tryptophan or peptone broth is the medium used for indole test (Color Photo 5).

Procedure : The test is performed by inoculating the medium with bacteria, incubating at 37°C for 24–48 hours. Then, 5 drops of Kovac's reagent containning amyl or isoamyl alcohol, *p*-dimethyl amino benzaldehyde, and concentrated hydrochloric acid are added to the inoculated medium.

Positive test is indicated by formation of a red ring at the surface of the medium.

No color change indicates a negative test.