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BOVIN MASTITIS: PREVALENCE, RISK FACTORS AND MAJOR PATHOGENS IN THE SIDAMO ZONE SNNPRS, ETHIOPIA.

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ABSTRACT: ***A*** *cross sectional study was conducted from September 2010 to August 2011 on small holder dairy farms to determined the bovine mastitis prevalence, risk factors and major pathogens in urban and peri-urban areas of Sidama Zone, Southern Ethiopia. 161 Farmers addressed through using semi-structured questionnaires regarding the different potential risk factors and 96 dairy cows used for clinical, subclinical and laboratory examination. Both subclinical and clinical bovine mastitis were observed in the study areas; the more prevalent was subclinical mastitis 42.7%. 2(2.08%), and the lower was clinical mastitis (2.08%). In this study, there was a significant differences (P<0.05) between lactating cows at different location, breed and lactation stages; however, there was no significant differences (P>0.05) between lactating cows with parity and age. Of 384 quarters examined, 5(1.3 %) were blind teats and 71(18.4%) quarters showed evidence of infection of mastitis. Although the dairy farm owners have some awareness about mastitis, still now it's continuing as devastating disease. The milk samples collected from california mastitis test positive were subjected to microbiological examination and then many bacterial species were isolated such as: Staphylococcus aureus (21.1%), and Staphylococcus intermedius (17.3%) followed by Staphylococcus epidermidis (13.5%), klebsiella (13.5%), Micrococcus species (9.6%), Staphylococcus hyecus (7.7%), Aracnobacterium pyogen (5.7%), Escherichia coli (3.8%), Enterobacter species (3.8%) and Bacillus cereus (3.8%). This study showed that mastitis is the problem of dairy cows in the study areas; this disease associated with herd management system, drainage, floor types and level, milking procures and manure removal schedule and the major isolates that were contagious pathogens. Therefore, hygienic milking practice, milking order, hygienic of the utensils used for milking practice, proper manure removal and housing and management improvements are some of the important procedures which need attention while the prevention of the disease. Before treatment, susceptibility testing for the isolated microorganisms is very vital in controlling strategy.*

KEYWORDS*: Bacteria isolation, Bovine Mastitis, CMT, Risk factors, Sidama Zone Ethiopia.*

INTRODUCTION

In Ethiopia, the livestock sector has been contributing a considerable portion to the economy of the country, and still promising to the economic development of the country. It is important that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people. The country has a largest livestock population of any African countries with an estimated 56.7 million cattle, 58.6 million sheep and goats, 10 million equines,

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1.2million camels, and more than 56.8 million chickens and immense bee and fishery resources. In Southern Nations Nationalities and People Regional States (SNNPRS), the total cattle population is estimated at about 8.8 million. Nearly all the cattle population (98.41%) is found in rural areas while a small proportion (1.59%) is found in urban (CSA, 2003).

The livestock diseases which potentially infect and affect the wellbeing of livestock population among those mastitis, is the common and costly disease causing loss in milk yield and treatment cost for dairy farmers, its prevalence ranges from 1.2 to 46.6% in different areas of Ethiopia (Abdella, 1996; mokonen *et al*., 2006). The average cost of a case of mastitis due to antibiotics used, milk discarded, reduction in quality and quantity of milk produced by a cow (Blowey, 1990) and the economic loss due to clinical and subclinical mastitis per lactation in Ethiopia is 270 ETB (Mungube *et al*., 2005). Because of its multifactorial causation, Mastitis is considered as the most complex disease (Harmon, 1994).

Mastitis, inflammation of the mammary gland (Fekadu, 1995; Mekonnen *et al., 2005*), is classified as clinical and sub clinical based on aetiopathological findings and observation, clinical mastitis is further classified as per acute, chronic and gangrenous mastitis. Subclinical mastitis refers to inflammation of the mammary gland in the absence of visible gross lesion in the udder or its secretion with the presence of pathogenic microorganisms and usually high number of somatic cells in the milk (Harmon, 1994; Radostitis *et al*., 2007; DACA, 2006). Mastitis, as a disease, has received little attention; especially the sub clinical form of mastitis (Mekonnen *et al*., 2005; sori *et al*., 2005).

Even though, many researches of mastitis done for many years mastitis remains the most economically important disease worldwide for dairy industries and public health by serving as a vehicle in the spread of diseases like tuberculosis, staphylococcal food poising and brucellosis (Radostits *et al*., 2007). The researches work showed that on the average the affected quarter suffers a 30% reduction in productivity and affected cow a 15% loss its production (Blood, 1989). The total annual national milk production in Ethiopia ranges from 797,900 to 1,197,500 metric tons of raw milk, out this from 85% to 89% is contributed from cattle. However, the national demand for milk and milk products in the country is not satisfied. Among the many reasons which lowered the annual national milk yield and quality mastitis is one of the most important problem in the dairy industries of Ethiopia (FAO, 2003; Nesru, 1999). In Ethiopia, mastitis has not given an attention as a disease particularly the subclinical form, only been concentrated on the treatment of clinical cases (Nesru, 1999).

The majority of microorganisms that are responsible for mastitis and spoilage of milk could be *Staphylococcus aureus*, *Streptococcus agalactiae, Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis*, coliforms (*Escherichia coli*, *Klebsiella* species and *Enterobacter aerogenes), Serratia, Pseudomonas, Proteus* species, environmental *Streptococci, Enterobacte*r species (Quinn *et al.,* 2002), and they are responsible for diseases like tuberculosis, streptococcal intoxication, colibacillosis, streptococcal sore throat and brucellosis in human (Radostits *et al*., 2007). As most infectious diseases, generally mastitis risk factors depend on three components;

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exposure to microbes, cow defense mechanism, environmental and management factors (Quinn *et al*., 2002).

Mastitis is an important factor that limits dairy production due to its heavy financial losses involved and the existence of latent infections characteristics. A few researches have been done concerning on the status of bovine mastitis in Sidamo and Gedio zones unlike that of other areas of the country (FAO, 2003). These study area are the most known milk shade areas in the country. Therefore, the objectives of this study are to determine the prevalence of bovine mastitis, assess the potential risk factors and isolate the Pathogens in smallholder lactating dairy cows.

MATERIALS AND METHODS

STUD AREA

The study was conducted in urban, peri urban and rural areas of Sidamo zone SNNPRS, Ethiopia. Sidamo zone located at North 5′ 45″ and 6′ 45″ Latitude and East, 38′ and 39′ longitude (CSA, 2014). From selected districts of Sidamo zone a total of 15 peasant associations were participated in the survey study, and also the laboratory tasks of this zone were done in Hawella Tula, A/chuko and A/wondo districts and two peasant associations were selected from each districts.

Sidamo zone has a total area of 10,000 km^2, of which 97.71% is land and 2.29% is covered by water body (Hawass Lake and Logita falls). 4% coverage area of the Sidamo zone is ranging from 1500masl to 2500masl elevated with mean annual rainfall of this area varies between 1200 mm and 1599 mm, with 15 °C to 19.9 °C average annual temperature, where as the hot climate zone covers 30% of the total area, and elevated from 500masl to 1500masl with mean annual rainfall of 400 mm to 799 mm, and the mean annual temperature ranges from 20 °C to 24.9 °C. And 16% coverage area is elevated from 2500masl to 3500masl, cool climate condition with rainfall ranging from 1600 mm to 1999 mm and has a mean annual temperature of 15 °C to 19.9 °C. The livestock population of the study areas is 2,131,224 cattle, 455,052 sheep, 267,039 goat, 50,143horse, 10,008mule, 69,941donkeys, 1,785,141poultry and 96,114 beehives (CSA, 2014).

Study Animals

The study animals were lactating smallholder dairy cows of different management conditions with different breeds, age groups, lactation stages and parity were examined as study population. A total 384 milk samples collected from 96 lactating cows from three dairy potential districts (Hawella Tula, A/chuko and A/wondo).

Study Design

Cross sectional study was conducted during the study period; the study areas were purposively selected to handle mastitis occurrence assessment and associated risk factors. The lactating cows and quarters level examination based on clinical manifestations for clinical mastitis and indirect test that was California mastitis test and culture(CMT) for sub clinical mastitis and bacteriological examination(culture, stain, and biochemical tests on the pure isolates)to isolated the pathogens.

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Questionnaire Survey

The survey was conducted using semi-structured questionnaires regarding the different potential risk factors like breed, age, parity, lactation stage, previous history of mastitis, housing conditions, milking hygiene and general management conditions.

The dairy farms were physically inspected for cleanness, handling, milking procedures and other factors associated with mastitis. The udder was examined clinically, using visual, then through palpation to detect possible swelling, pain, and disproportional symmetry, blindness of teats and discoloration of milk for the presence of mastitis according to (Quinn *et al.,* 2002).

Clinical and Subclinical mastitis

Clinical mastitis was diagnosed on the basis of visible or palpable sign of inflammation together with change in consistency and color of milk secreted. The California mastitis reagent was used to screen the cows with sub clinical mastitis. Milk samples collection was performed according to the procedures recommended by national mastitis council (NMC, 1990). The result of the test was indicated on the basis of gel formation. The interpretation (grades) of the CMT was evocated and the results graded as 0 for negative and trace +1, ++2 and +++3, for positive (Quinn *et al.,* 2002; NMC, 1999).

Milk Samples Collection, Transportation and Storage

The milk sample was taken from cows not treated with either intra mammary or systematic antimicrobials agents. Milk samples were collected by a standard milk sampling techniques (NMC, 1999). For good collection of sample the teat were wiped thoroughly with 75% ethyl alcohol. Approximately 10 ml of milk were collected in to a sterile test tube after discarding the first 3 milking stream. The milk sample then held in an icebox for transportation to the laboratory. In laboratory samples was cultured immediately or stored at +40C for a maximum of 24 hour until inoculated on a standard bacteriological media ((Biru, 1989; NMC, 1999). After collection of the CMT positive milk sample from different quarters, all CMT positive samples were clearly labeled with the cows identification number using permanent marker on the test tube. And then transported with icebox to the laboratory and processed immediately (Quinn *et al*., 2002). The isolation and identification of pathogenic bacteria performed at Woliata Sodo Regional Veterinary Laboratory.

Bacteriological isolation

Bacteriological examination was done according to the NMC (1990) and National Committee for Clinical Laboratory Standards (NCCLS) (1997). A loopful of milk sample was streaked on tryptose blood agar base enriched with 5% blood agar (Oxoid, UK) and MacConkey Agar using the quadrant streaking method for each quarter. Plates were incubated aerobically at 37°C for 24hrs - 48hrs. The plates were examined for gross colony morphology, pigmentation and haemolytic characteristics after 24 - 48 hrs of incubation.

Identification of bacteria to the species level was carried out based on their colony characteristics, Gram's staining and morphological characteristics, growth on MacConkey agar, catalase, urease and oxidase tests, hydrogen sulfide (H2S) production, indole, methyl red(MR), and Voges-Proskauer (VP) reaction, citrate utilization, oxidation-fermentation test, motility, TSI(Triple Sugar Iron Test) and different carbohydrate testes.

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Data Collection and Analysis

SPSS software or descriptive statistics were used to summarize the data which is collected through survey, CMT, clinical inspection, pathogenic bacteria isolation results. And the data entered into data base management software Microsoft Excel computer program. The prevalence of mastitis was calculated using percentage values, and the possible association of the disease with different risk factors was analyzed by using chi-squared (x2) test.

RESULTS

Survey Analysis

Based on the survey study, out of the 161 respondents, males 106 (65.8%) and females 55 (34.2%) were recorded. Male farm owners were the prominent in the study area. As observed from the educational level of farm owners or attendants were primary 92(57.1%), secondary 47(29.2%), illiterate 20(12.4%) and University 2(1.2%).

Housing

The floor of dairy farms in the study areas were 48.4%, soil 21.7% wooden 16.1% concrete and 13.7% stony types, and the floor level types of the dairy were 49% leveled and 51% sloppy. The majority of dairy farms their houses roofed with grass (55%) and 42% houses roofed with metal sheet; the higher 66% of the dairy farms their houses walled with concrete and followed by 32% walled with other housing materials and the rest 2% walled with mud. From the survey regarding to manure removal the majority of dairy farms remove their manure weekly (98%) and some of remove their manure daily (2%).

Milk Production

The dairy farm owners who had a usually milk recording habit were 13%, sometimes record 6.2% and not record 80.7%; the maximum milk yield recorded 18liters and 0.5 liter was minimum. The survey also revealed that the dairy farm owners assumed that cows with higher milk production related to mastitis were 52.8%, whereas mastitis related to low milk producers cows and both of the higher and low milk producer cows were 6.8% and 40.4% respectively. 24.2 % owners responded that younger animals more exposed to mastitis than older animals, 22.4%.

Milking Practices

The dairy farms which washed the udder of the lactating cows always before milking were 64.6%, out of these dairy farms only 25.5% farms were practiced dry udder after washing plus74.5% were not practiced dry udder after washing whereas 35.4% dairy farms were not wash udder while milking their lactating cows. The dairy farms that practiced udder drying using the same towel were 80.1%, the remaining percentage separated towel were used to dry udder. Generally, the study indicated that 53.4% dairy farms owners not followed the milking procedure, but 46.6% dairy farms owners followed the milking procedure.

Mastitis Occurrence

51.9% of the dairy farms have reported the presence of previous history of mastitis, and they observed the clinical mastitis, but the remaining 49.1% dairy farms didn't have mastitis in their

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previous history. The survey assessment revealed that the occurrence 75.8% of clinical mastitis, 67% tick infestation, and 76.4% presence of abortion in previous history in the dairy farms.

Prevention and Control

Regarding to farm owners awareness to prevention and control strategies of mastitis in their dairy farms awareness some of them use drugs, like penstrips 19.9%, oxytetracycline 13.7%, Intramammary infusion 11.8% and mixed treatment options 6.2%, and 48.6 % of the dairy farms owners didn't aware to treatments. The dairy farm owners got services to treat their mastitic cows from veterinary clinic, 83.3%, and 16.7%, treat themselves. The drug sources for the farms were veterinary pharmacy, veterinary clinic and more than one source 2.5%, 26.1%, 9.9% respectively. The 65.8% respondents were free from ethno veterinary practice but the 34.2% were used traditional veterinary medicine in cure their animals.

Prevalence of Mastitis Associated with Risk Factors

Subclinical and Clinical Form Mastitis

From 96 lactating cows total 384 milk samples were collected and examined for the presence of mastitis that was 2 (2.08 %) and 41(42.7%) were positive for clinical and sub clinical mastitis respectively. The prevalence of subclinical mastitis in woredas: 58.06% in Alta wondo, 4.75% in Hawella Tula and 27.27% in Aleta Chuko. The study result indicated that there was statistical significance (P<0.05) between woredas (**Table 1**).

Table 1: The prevalence of mastitis based on different location and cow levels

Woreda	N	CMT Positive (subclinical mastitis)	Prevalence (%)	Clinical mastitis	Prevalence (%)
Aleta Wondo	31	18	58.06	1	3.23
HawellaTula	32	14	43.75	1	3.13
Aleta Chuko	33	9	27.27	-	-
Total	**96**	**41**	**42.71**	**2**	**2.08**

Pearson chi(x^2) = 6.215, p = 0.045

Prevalence of Mastitis by Breed

In this study area, the prevalence of mastitis associated with local breed was 33.3% and with cross breed was 58.33%. The prevalence of mastitis associated with breeds was found statistically significant (P<0.05) (**Table 2**).

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Table 2: Indicates the prevalence of the mastitis associated with breeds of lactating cows

Breed	NO. Examined	CMT Positive	Prevalence (%)	X^2	P-value
Cross	36	21	58.33		
Local	60	20	33.33	5.747	0.017
Total	96	41	42.71		

Prevalence of Mastitis by Age

The prevalence of mastitis was measured for different age groups of lactating cow young, 47.73% and adult, 38.46%. Even if the prevalence associated age groups found statistically insignificant with a P-value of 0.360, the prevalence was found higher in the young age group than the older age group (**Table 3**).

Table 3: Prevalence of mastitis based on different age groups

Dam's Age	NO. Examined	CMT Positive	Prevalence (%)	X2	P-value
2-6years(young)	44	21	47.73		
7-12 years(adult)	52	20	38.46	0.836	0.360
Total	96	41	42.71		

Prevalence of Mastitis by Parity

The prevalence of mastitis associated with parity in dairy farms were 33(48.53%), 7(29.16%) and 1(25%). in 1-3, 4-5 and greater than or equal 6 parity respectively. The prevalence of mastitis measure based on the parity of cows was. The study revealed show that there was no statistical significant (P>0.05) between mastitis associated with parity (**Table 4**).

Table 4: Prevalence of mastitis associated with parity in the dairy farms

Parity	No. Examined	CMT Positive	Prevalence (%)	X^2	P-value
1-3	68	33	48.53		
				3.253	0.197
4-5	24	7	29.16		
>/= 6	4	1	25.0		
Total	96	41	42.71		

Prevalence of Mastitis by Lactation Stage

The prevalence of mastitis on early, mid and late stage of lactation was (47.62%), (28.57%) and (57.56%) respectively. The result showed that the disease is significantly associated with stage of lactation (P<0.05) (**Table 5**).

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Table 5: Prevalence of mastitis based onstage of lactation

Lactation Stage	No. examined	Positive CMT	Prevalence %	X^2	P-value
Early	21	10	47.62		
Mid	42	12	28.57	6.619	0.037
Late	33	19	57.56		
Total	96	41	42.71		

Prevalence of Mastitis by Quarter Level

This study showed that the prevalence of mastitis in quarter level as left hind 19.8%, right hind 18.8%, right front 17.7% and left front 16.7% recorded. The left hind quarters shows the highest infection rate followed by the right hind (**Table 6**).

Table 6. Prevalence of mastitis at quarter level

Quarter	NO. Examined	Blind Quarter	CMT Positive	Prevalence (%)
Right front	96	-	17	17.7
Left front	96	1	16	16.7
Right Hind	96	2	18	18.8
Left Hind	96	2	19	19.8
Total	**384**	**5**	**70**	**18.23**

Culture Growth

The CMT positive samples were cultured and 74.28% grown and 25.71% not growth on the culture (**Table 7**).

Table 7: Bacterial culture from CMT positive indicated both positive and negative results

Test used	No. of sample	Percentage (%)
Growth on culture	52	74.28
No growth on culture	18	25.71
Total	70	100

Isolated Bacterial Species

The bacteria species isolated from CMT positive samples that growth on culture are *Staphylococcus aureus, Staphylococcus intermidius, Staphylococcus epidermidis, Staphylococcus hyecus, Micrococcus* species, *E.coli, klebsiella, enterobacter spp, aracnobacterium pyogen, Bacillius cereus* (**Table 8**).

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Table 8: Isolated bacterial species from CMT positive

Bacterial isolates	Isolates Frequency	Isolation %
Staphyloccus aureus	11	21.1
Staphylococcus intermedius	9	17.3
Staphylococcus epidermidis	7	13.5
Staphylococcus hyecus	4	7.7
Micrococcus species	5	9.6
Escherichia coli	2	3.8
Klebsiella	7	13.5
Enterobacter species	2	3.8
Aracnobacterium pyogen	3	5.7
Bacillus cereus	2	3.8
Total	**52**	**100**

DISCUSSION

In this study areas male 106(65.8%) cover majority of the dairy farm operations than female 55 (34.2%); the primary school 92(57.1%) educational status of the dairy farm owners was the prominent and followed by secondary school, illiterate and university 47(29.2%), 20(12.4%) and 2(1.2%) respectively. The majority lactating cows in the dairy farms were located in the per-urban, 39.1% and followed by urban and rural 33.5% and 27.3% respectively.

The present study revealed the dairy farms owners awareness on mastitis, the majority have information about the disease how it came to the picture in their farm that said this disease associated with lactating cow such as (52.8%) milk production and (46.6%) milking procedures, management system (46% extensive system, 28% intensive and 26.1% semi intensive system), house floor types(48.4% soil, 21.7% wooden, 16.1% concrete and 13.7% stony) and level(49% leveled and 51% sloppy), removal of manure(2% daily, and 98% weekly), (51.9%) knowing previous mastitis occurrence, (15.5%) abortion occurrence, (32.9%) the present of tick infestation, (24.2%)the presence of teat lesion and (83.2%) use veterinary clinic and (34.2%) ethinoveterinary practice to cure their mastitic cows. In Ethiopia the efforts have been concentrated on the treatment of clinical cases, but the subclinical forms of mastitis received little attention (Almaw *et al*., 2008) but the reality that found on the ground of this study the subclinical mastitis prevalence is more prevalent than the clinical one, if so the strategy of bovine mastitis prevention should be reestablished using the findings of the different studied did in the country.

The result of this study showed that the overall prevalence of mastitis in lactating cows (cross bred 58.33% and local bred 33.33%) in Sidamo zone was 42.71%, which agreed with the findings of Fekadu (1995) in Caffa valley in Northern Ethiopia (39.65%) and Kerro Dego and Tareke (2003) in Southern Ethiopia (40.40%) on bovine mastitis(Table 1). However, it was higher than the

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findings of Biffa (1994), 33.0% and Biffa *et al*.(2005) in Southern Ethiopia, 34.9%, but lower than the findings of Tolossa(1987) *around Kallu Province*, Ethiopia 53.5%, Biru(1989) in Ethiopia, 63.4%, Haile(1995) *in South Wollo*, Ethiopia 53.35%, Zerihun(1996) in Ethiopia 68.1%, Workineh *et al*.(2002) in two major Ethiopian dairies 59.7%, Sori *et al*.(2005) in and around Sebeta, Ethiopia 52.78%, Mekibib *et al.(*2010) in Holeta town in Centeral Ethiopia 71.05% and Birhanu *et al*.(2013) in Oromia reginal state,South Eastern Ethiopia, 66.6%. The variability in the prevalence of bovine mastitis between findings could be suggested the complexity of the disease which involves interaction of several factors, mainly the difference in management of the farms, breeds considered, husbandry system, environment, factors related to causative agent, variation in veterinary service coverage and awareness of the owner toward the disease, and technical know-how of the researchers.

The prevalence of mastitis associated with breeds, cross bred 58.33% and local bred 33.33%, was found statistically significant (P<0.0.5) (Table 2). Breed difference can play a vital role in the prevalence of different diseases. In this study, the prevalence of mastitis in crossbred cows was statistically higher than that of local cattle. This finding is comparable with report of other studies such as Almaw *et al*. (2009) in and surrounding of Gondar town, Sori *et al*. (2005) in and around Sebeta, Ethiopia and Junaidu *et al*. (2011) at Sokoto metropolis. This variation of mastitis prevalence in breeds level could be that the disease is associated with the lactating cow's milk yield that recently stated high yielding cows are more susceptible to mastitis than low-yielding ones(Radostits *et al*., 2007). And also the survey result agreed with this idea; the dairy farm owners responded that the prevalence of mastitis with higher milk producer cows, low milk producers cows and both (higher and low milk producer cows) were 52.8%, 6.8% and 40.4% respectively. In addition to these factors, Stress associated with a high milk yield may upset the defense system of the animal. 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) and due to less efficacy of phagoacytic cells in higher yielding cows associated to dilution(Schalm *et al.,* 1971). And also may be influenced by some inheritable characteristic such as capacity of milk production teat characteristic and udder conformation (Abaineh, 1997).

Of the total 96 lactating cows a 384 samples utilized for the study purpose, the results this study showed prevalence of sub-clinical and clinical mastitis as 41(42.7%) and 2(2.08%) respectively. The subclinical mastitis was highly prevalent (58.06% in Alta wondo, 4.75% in Hawella Tula and 27.27% in Aleta Chuko) than the clinical mastitis(3.23% in Alta wondo, 3.13% in Hawella Tula and zero percent in Aleta Chuko) in the study areas; they showed the statistical significance between the districts(P<0.05) (**Table 3**). This finding was slightly agreed with the result of Nesru (1999) in Addis Ababa, central Ethiopia 5%, Bitew *et al*. (2010) in Bahir Dar and its environs, 3% and Nibret *et al*.(2012) in Hawassa, Southern Ethiopia 4.9% and Benta and Habtamu (2011) in Batu and its environments, Ethiopia 5.3% on prevalence of clinical mastitis, but lower than the reports of Sori *et al*.(2005) in and around sebeta, Ethiopia 16.11%, Biffa (1994) in southern Ethiopia 15.1%, Radostits and Blood (1994) 10%, Mekibib *et al*.(2010) in Holeta town in Centeral Ethiopia 10%, Birhanu *et al*.(2013) in Oromia reginal state,South Eastern Ethiopia 12.1% and Biffa *et al.(*2005) in Southern Ethiopia 11.9%. The Sub-clinical mastitis has been found to be higher than the reports of Nesru (1999) in Addis Ababa, central Ethiopia 32.2%, Bitew *et al*.

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(2010) in Bahir Dar and its environs 25.2%, Nibret *et al*.(2012) in Hawassa, Southern Ethiopia 30.6%, Mekibib *et al.(*2010) in Holeta town in Centeral Ethiopia (34.8%), Sori *et al*.(2005) in and around sebeta, Ethiopia (36.67%), Birhanu *et al*.(2013) in Oromia reginal state,South Eastern Ethiopia (54.5 %), Biffa *et al.(*2005) in Southern Ethiopia 23.0% , but the same with report of Benta and Habtamu (2011) in Batu and its environs, Ethiopia 40.6%. This could be due to difference in management system in that study areas the most applied farming system was extensive (37.3%) and followed by intensive (24.2%) and semi intensive (23.6%). Environmental bacterial mastitis were higher in prevalence, due to poor housing facilities which predispose the accumulation of faeces on cows which will increase the rate of exposure of the teats and udder to the pathogens(Radostits *et al.*, 2007).

The prevalence of mastitis that associated with age groups of lactating cows in the study site was 47.73% in young and 38.46% in adult(Table 3), and the prevalence of mastitis with parity was 48.53% in 1^{st} - 3^{rd} parity groups and followed by 29.16% in 4^{th} -5^{th} parity group but lower in 6^{th} or more parity groups, 25.0% (Table 4). Even though the prevalence of mastitis in the young and 1^{st} -3^{rd} parity group lactating cows were higher than the adult and the 6^{th} or more parity group cows, there were no statistically significant among the age and parity groups ($p>0.05$). The present study is in agreement with finding of G/Michael *et al*.(2013) in and around areka, Southern Ethiopia based on parity group mastitis prevalence that was statistically insignificant. The decreased prevalence of the mastitis with age and parity reported in the present finding is not agree with finding of Biffa *et al*. (2005) who reported that increased prevalence of mastitis with age and parity. The present finding from Biffa *et al*. (2005) who reported that increased prevalence of mastitis with age and parity, because due to the farmers management system actually applied up on their different age group lactating cows is different that their young cow and that has less parity grazed freely in extensive system whereas the adult cow and that has more parity fed in zero grazing or intensive system this happen because of the owners fear to their adult cows might be damaged or fall down in the field, they care more because they assumed they are productive and also not effective in grazing.

The stages of lactation indicated that statistically significant in the study site 47.6%, 28.6% and 57.3% in early, mid and late lactation, respectively, ($p<0.05$) (Table 5). This result is coincided with G/mechael *et al*. (2013) and Biffa *et al*. (2005) who reported lactation stage has significant effect on the prevalence of mastitis in Ehiopia. Early stage and the late stage of lactation were the most susceptible stages. The mid lactation was lower. These results are consistent with previous findings (Hughes, 1960; Kehrli and Shuster, 1994; Radostits and Blood, 1994). And also it's similar with the report of Biru (1989) and those at early and late stage of lactation are at a great risk to mastitis than mid stage of lactation. This could be due to the delayed diapedesis of neutrophils to mammary gland in recently calved cow and at late lactation there is decrement of neutrophil concentration when the cows reach to dry off (Workineh *et al*, 2002).

A total of 384 quarters were examined, of which 70(18.23%) were positive for mastitis while 5(1.30%) were blind (**Table 6**). The prevalence of mastitis associated with quarter, left hind (LH) showed the highest rate of infection (19.8%) followed by the right hind (RH) which is 18.8%. The quarter prevalence of mastitis (18.3%) found in this study was comparable with the finding of

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Almaw (2004) who reported the prevalence rate of 17.9 % in and around Bahirdar town, Ethiopia and lower than Bachaya *et al.*(2011) reported in Pakistan 35.25%, and Fadlelmoula *et al.* (2007) who reported in Germany 27.57%. As compared to the others the left hind quarters were affected with the highest infection rate (19.8%). The right hind quarters were the second with an infection rate of 18.8%. Thus, this study revealed that the hind quarters have higher infection rate than the right quarters. This might be due to the high production capacity of the hind quarters (Radostitis and Blood, 2007) and the high chance of getting fecal and environmental contamination (Sori *et al., 2005).*

From a total 70 CMT positive samples 74.28% was grow and the remained 25.71% not grow on the culture (Table 7). Out of the 70(74.28%), the gram positive cocci bacteria were 36(69.23%), gram negative rods 11(21.15%) and 5(9.62%) gram positive rods. The bacteriological analysis result obtained from this study showed that 74.28% were bacteriologically positive which is compared with zeryehun *et al.*(2013) report 67.8%.
With regard to the bacteriological analysis of milk samples, the study revealed that from the 70 CMT positive milk samples the most prevalent bacteria isolates were *Staphyloccus aureus (21.1%), Staphylococcus intermedius (17.3%), Staphylococcus epidermidis(13.5%), Klebsiella(13.5%), Micrococcus species (9.6%) Staphylococcus hyecus(7.7%)* and *Aracnobacterium pyogen(5.7%),* where as the lower isolates were *Bacillus cereus(3.8%), Enterobacter species (3.8%)* and *Escherichia coli(3.8%)(Table 8).*

In this finding, *S. aureus* was the predominant pathogen that constituting 21.1% of all isolates. The high level isolation of *S. aureus* (21.1%) in this study almost coincided with the finding of zeryhun *et al*.(2013) reported 28.7% and the predominance and primary role of *S. aureus* isolate in bovine mastitis has also been reported in other studies (Atyaib *et al.*, 2006; Fadlelmoula *et al.*, 2007; Mekbib *et al.*, 2010), but it's lower in comparative with the finding of Birhanu *et al*.(2013) in Oromia reginal state,South Eastern Ethiopia reported 35.71% and Lakew *et al.* (2009) who reported in Asella, southern Ethiopia 39.4%. This study also not harmony with the finding of Bishi (1998) and Edwards *et al.* (1982) who found CNS as the predominant species from urban and peri-urban production system in Ethiopia and Bolivia, respectively. *S.aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process (Radostitis *et al.*, 1994). Thus, the organism has been assuming as a major importance as a cause of bovine mastitis.

In this study, *E. coli* (3.8%) was the lowest of all isolated bacteria from mastitis positive samples, but *E.coli* was matched with reports of Molalegne *et al*. (2010) who reported 2.5% and Mengistu (1986) who found 3.14% in Bahrdar, Ethiopia; and higher than the finding of Sori *et al*.(2005) in and around Sebeta, Ethiopia. The present findings, *staphylococcus intermedius* (17.3%) is higher than Brihanu *et al*.(2013) who reported (7.14%) ,but lower than the 38.4% report of Argaw and Tolosa (2008). *S. epidermids*, Coagulase negative bacteria, which contribute about 13.5% of the isolates and agreed with the report of Sori *et al*. (2005) in and around Sebeta, Ethiopia 14.93%.
S. hyecus is a gram negative staphylococcus rarely a causative agent of mastitis in cattle (Carter and Cole, 1990); in this study it scored 7.7%. The dairy owners wash the udder every time before

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milking 64.6% and the remained portion was not wash 35.4%, only 25.5% owners practiced dry after udder washing, but the large portion 74.5% no habit drying after udder washing. Therefore, the infection rate of S. hyecus might be due to the fact that they easily transmitted during milking via the teat cups and milker's hands.

The 9.6% isolated *Micrococcus* species in this study was comparable with the finding of Bedada and Hiko (2011), Mekonnen *et al*. (2005) and Birhanu *et al.(*2013)who reported 5.6%, 10.2% and 7.14% respectively. But it was much lower than Ameni *et al.* (2003) who reported 26.67% in different part of Ethiopia. Such differences and similarities may result from management system and ecological difference in agent.

klebsiella 13.5% and *enterobacter spp* 3.8%, in the current study lower to similar studies with the study of Garedew *et al*. (2012), Alehegne(2004) and Tassew and Seifu(2011) in Ethiopia. The isolation of these bacteria indicated due to poor and unhygienic bedding condition in the majority of farms and absence of teat dipping and disinfection practices in the current study. These practices have been known as critical components of mastitis prevention and control program in dairy herds (Galton *et al*, 1986). The result this work *aracnobacterium pyogen* isolation, *5.7%*, was higher than finding of G/micheal *et al.*(2013) who reported 1% in Ethiopia and this bacteria can affect both immature and lactating gland lead to abscess formation which named as Summer Mastitis(Awale *et al*,2012) . The 3.8 % *Bacillus* cereus isolated were similar with the report of Birhane *et al.(*2013), 5.71%. Radostits *et al.(*2007) reported that *Bacillus* species only occasionally mastitis causing pathogens. The infection is associated with contamination of teat injures and surgery. The survey findings also support to this that showed the present of tick infestation 32. 9% and teat lesion 24.2% were responsible for the bovine mastitis. The level of infection can be high during the dry period following the use of dry cow therapy preparation which may have been contaminated with the organisms.

CONCLUSIONS AND RECOMMENDATIONS

The study has shown that mastitis is still a major problem to small holder dairy farms and the findings suggested that mastitis may be a limiting factor in increasing milk production. The sub clinical mastitis was found to be a devastating problem and it occurred in both breed of lactating dairy cattle on urban, periurban and rural areas. Major exposing factors that lead mastitis were breed, and lactation stage, management problems, age and parity. The staphylococcus bacteria were the predominant one that causes mastitis.

Therefore, based the above conclusions the following point forwarded:

- So, the preventive and control strategy of mastitis should have established that focusing up on awareness rising of the dairy farms owners to follow the key factors of mastitis such as good herd management, washing milkers hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method, teat dipping before and after milking and treating clinical cases at early stage.

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- Adequate housing with proper sanitation and regular screening for early detection and treatment, culling of older cows with repeated attacks and follow up of chronic cases by veterinarians.
- Susceptibility testing of the isolated microorganisms is recommended before treatment.

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