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## Research article

## Study of Prevalence, Associated Risk Factors and Causative Bacteria of Bovine Mastitis in Ethiopia -

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## Abstract

**Bovine** mastitis has a negative impact through economic losses in the dairy sector across the globe. A cross sectional study was carried out from September 2015 to July 2016 to determine the prevalence of bovine mastitis, associated risk factors and isolation of major causative bacteria in lactating dairy cows in selected districts of central highland of Ethiopia. A total of 304 lactating cows selected randomly from five districts were screened by California Mastitis Test (CMT) for subclinical mastitis. Based on CMT result and clinical examination, over all prevalence of mastitis at cow level was 70.62% (214/304). Two hundred fourteen milk samples collected from CMT positive cows were cultured for isolation of major causative bacteria. From 214 milk samples, 187 were culture positive and the most prevalent isolates were *Staphylococcus aureus* 42.25% (79/187) followed by *Streptococcus agalactiae* 14.43% (27/187). Other bacterial isolates were included Coagulase Negative *Staphylococcus* species 12.83% (24/187), *Streptococcus dysgalactiae* 5.88% (11/187), *Escherichia coli* 13.38% (25/187) and *Enterococcus faecalis* 11.23% (21/187) were also isolated. Moreover, age, parity number, visible teat abnormalities, husbandry practice, barn floor status and milking hygiene were considered as risk factors for the occurrence of bovine mastitis and they were found significantly associated with the occurrence of mastitis ( $p < 0.05$ ). The findings of this study warrants the need for strategic approach including dairy extension that focus on enhancing dairy farmers' awareness and practice of hygienic milking, regular screening for subclinical mastitis, dry cow therapy and culling of chronically infected cows.

**Keywords:** Bovine mastitis; Bacteria; Ethiopia; Prevalence; Risk factors

## INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and still promising to rally round the economic development of the country [1]. It is eminent that livestock products and by products supply provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people [2]. Even though Ethiopia is the most populous country in cattle than any African country; the per capital milk consumption was 16 kg, which was lower than other countries in the region [3]. This is partly due to the low genetic milk production potential of the indigenous zebu cattle [2]. To increase milk production cross breeding of indigenous zebu with exotic breeds particularly with Holstein Friesian is widely practiced which resulted in a larger portion of the dairy cattle population. However, this market oriented dairy production, a rapidly growing system in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders [4].

Mastitis is one of the most important disease affecting dairy cows and it is a multi-factorial disease with worldwide distribution which incurs serious economic losses to dairy industry [5]. The disease results in decreased production, discarded milk and medical treatments as well as a higher level of premature culling of affected animals [6]. Milk and milk by-products are considered to contribute to the social and economic development in rural areas where the dairy production from cattle is one of the major sources of income in many households [7]. Dairy products also provide essential food and nutrition for people in these areas [8]. A number of previous reports from different part of Ethiopia indicated that mastitis is a serious problem in dairy industry [9]. Bovine mastitis can reduce milk yield, increase culling rate, incur treatment cost, and occasionally result in death from severe infection [2]. Moreover, mastitis had been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and to cause culling of animals at an unacceptable age [8]. Generally, as with most infectious disease, mastitis risk factors depends on three components that is exposure to the microbes, cow defense mechanism and environment and management factors [10]. The economic loss due to the disease is considerable and can be crucial, especially for small-scale dairy farmers in Ethiopia. Therefore, the objectives of this study were to determine the prevalence of bovine mastitis, isolate the major bacteria that cause bovine mastitis and to determine the various risk factors associated with the occurrence of mastitis in Ethiopia.

## MATERIALS AND METHODS

### Animal characteristics

The crossbred dairy cows (Borana  $\times$  Holstein breed) found in the study area is managed under extensive system as a source of milk, meat and drought power. Small holder dairy farms in most areas are housed always and provided feed in their stall.

### Study areas

This research was conducted in central highlands of Ethiopia that fall in the administrative territory of Oromia National Regional State. So, Oromia Special Zone of Surrounding Addis Ababa is found in the central part of the Oromia Regional State, surrounding the capital city-Addis Ababa. For the purpose of this study five (Holeta, Menagesha, Burayu, Sululta and Sebeta) major towns were selected based on number of commercial dairy farms [2].

### Study population and husbandry practice

Study animals were cross breed lactating dairy cows owned by government and other commercial dairy farms in central highland of Ethiopia. The animals were often managed under both semi-intensive and intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and agricultural by-products and some are maintained usually in separate stalls, a short distance from each other in a house.

### Study design and Sample size determination

Cross-sectional study was conducted from September 2015 to June 2016 in lactating cross breed dairy cows in the study area. The sample size was determined at 95% confidence interval, 5% precision and from previous studies in the study area by [11] with an expected prevalence of 34.4%. Thus, the sample size was calculated based on formula of [12] and the minimum sample size was 346 animals. However, due to the lack of cooperation in some farm owners, only 303 lactating dairy cows were included for this study.

### Study methodology

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



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

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

**Sampling method and Sample handling:** Milk samples were collected by standard milk sampling techniques from all lactating cows with clinical and subclinical mastitis. To reduce contamination of the teat ends during sample collection, the near teats were sampled first followed by the far once. Approximately 10 ml of paired milk was collected from each quarter (one for CMT and one for bacteriological examination) into labeled sterile screwed cap universal bottle after discarding the first three milking streams. Samples were placed in ice box and transported to the Animal Biotechnology Research Laboratory under National Agricultural Biotechnology Research Center and processed as soon as possible without any delay [13].

**California Mastitis Test (CMT):** Subclinical mastitis cases were diagnosed based on CMT results and the nature of gel formation (milk and CMT reagent), which shows the presence and severity of the infection [6]. Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT. From each quarter of the udder, a squirt of milk sample was dropped in each of the strip cups on the CMT paddle and an equal amount of CMT reagent was added to each cup and mixed gently. The test result was interpreted based on the thickness of gel formed by CMT reagent and milk mixture and scored as 0 (negative), T (trace), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Finally, quarters with CMT score of 1 or above were judged as positive for sub-clinical mastitis; otherwise negative [13].

**Bacteriological isolation and identification:** Milk samples from subclinical quarters were bacteriological examined according to the procedures employed by [13]. Briefly, before inoculating into primary culture medium, the milk samples were centrifuged so as to increase the bacterial load. A loop full of centrifuged mastitic milk sample was taken from each infected quarter and inoculated separately on to blood agar base enriched with 5% ovine blood using quadrant streaking method. The inoculated plates were incubated aerobically at 37°C for 24 to 48 hours, after which presence or absence of bacterial growth, colony morphology, color and hemolytic characteristics were recorded on primary culture. Prior to further biochemical tests, the isolated bacteria on blood agar were sub-cultured into nutrient agar. Each culture was subjected to gram staining to determine their shape, and gram reaction. Catalase test using 3% Hydrogen peroxide ( $H_2O_2$ ) was performed to identify catalase positive and catalase negative bacteria. Mannitol Salt Agar (Oxiod, UK) and purple base agar (Difco) with 1% maltose were used to differentiate *Staphylococcus* species and incubated at 37°C and examined after 24-48 hours for mannitol and maltose fermentation respectively. Tube coagulase test using rabbit plasma was used to identify the coagulase positive and

coagulase negative *Staphylococcus* species. Enterobacteriaceae species were identified using Oxidase test, SIM medium (Oxiod, UK) for sulfur production, indole test after addition few drops of kovacs reagent and motility test, TSI (Triple Sugar Iron) (Oxiod, UK) to detect sugar fermentation, sulfur and gas production, MacConkey agar (Oxiod, UK) for lactose fermentation and colony characteristics and Simmon's citrate agar (Oxiod, UK) to differentiate bacteria based on citrate utilization [14].

## Data entry and analysis

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

## RESULTS

### Prevalence of bovine subclinical mastitis and its associated risk factors

From a total of 303 samples collected from dairy farms of the study area and were screened by CMT, which yielded an overall prevalence of 70.62% that is 187 animals examined had infection in their udders as evidence of mastitis. This result showed that the association between the occurrence of mastitis in the selected cows and different potential risk factors. Accordingly, mastitis prevalence showed significant variation among different age groups, husbandry practice, visible teat lesion, parity number, barn floor status and milk hygiene ( $p < 0.05$ ) (Table 1).

Study animals were grouped into three as younger than 6 years, age range of 6 and 10 years and older than 10 years. From 50, 87 and 166 cows were examined with age in these age groups, 28 (56%), 50 (57.47%) and 136 (81.97%) were found positive for mastitis, respectively. The result showed that the prevalence of mastitis was significantly higher (81.97%) in cows older than 10 years followed by cows in the age range of 6 to 10 years and lowest in cows younger than 6 years. The statistical analysis also showed that there exist highly significant differences among the three age groups ( $p < 0.05$ ) in the occurrences of mastitis (Table 1).

Both parity number and visible teat abnormalities were found to be significantly ( $p < 0.05$ ) associated with the occurrence of mastitis. The highest prevalence of mastitis was observed 45 (90.00%) in cows with parity of more than 6, followed by less than 3 and between 3 and 6 parity (Table 1). The highest prevalence of mastitis was observed 99 (91.66%) in cows with two teat blind, followed by teat laceration/lesion one teat blind (Table 1).

Cows managed intensively ( $p < 0.05$ ) was highly susceptible to mastitis when compared to those managed semi intensively with respective number and percent of 79 (84.04%) and 135 (64.59%) among the totally examined cows, respectively (Table 1). The prevalence of mastitis was higher 196 (96.07%) in cows on farms with poor barn floor status condition and lower 8 (8.08%) in farms with good conditions barn floor status condition. Statistically, highly significant association was observed between mastitis and barn floor status of the farms visited ( $p < 0.05$ ) (Table 1).

Furthermore, mastitis prevalence was found to be higher 174 (93.04%) in only washed during milking and lower 40 (34.48%) in

**Table 1: Association between some selected risk factors and occurrence of bovine mastitis.**

Risk factors	Category	Number of examined	Number of positive	Prevalence (%)	X <sup>2</sup>	P - Value
Age group (Years)	< 6	50	28	56	22.63	0.000
	6-10	87	50	57.47		
	>10	166	136	81.97		
Parity number	< 3	67	48	71.64	11.86	0.003
	3-6	186	121	65.05		
	> 6	50	45	90		
Visible teat abnormalities	Normal	135	74	54.81	39.55	0.000
	One teat blind	46	31	67.39		
	Two teat blind	108	99	91.66		
	Teat laceration	14	10	71.42		
Husbandry practice	Intensive	94	79	84.04	11.82	0.000
	Semi-intensive	209	135	64.59		
Barn floor status	Poor	204	196	96.07	194.95	0.000
	Good	99	8	8.08		
Milk Hygiene	Only washed	187	174	93.04	118.36	0.000
	Washed and dried with towel	116	40	34.48		

washed and dried with towel (Table1). Statistical analysis showed the existence of significant ( $p < 0.05$ ) association between the occurrence of mastitis and milking hygiene (Table1).

Generally, in the present study, age, parity number, visible teat abnormalities, husbandry practice, barn floor status and milking hygiene were considered as risk factors for the occurrence of mastitis and they were found significantly associated with the occurrence of mastitis ( $p < 0.05$ ) (Table1).

In district wise, total cows examined in Farm A, Farm B, Farm C, Farm D and Farm E were 154,13,27,79 and 30, out of these 59.09 % , 84.61% ,100%, 72.15% and 93.33% cows were positive for mastitis, respectively (Table 2).

### Bacterial isolation

From a total of 214 milk samples cultured, 187 (87.38%) were culture positive. Analysis of bacteriological examination of milk samples was made to identify the main etiological agents involved in the bovine mastitis. Major causative bacterial were identified on the basis of their cultural, staining characteristics and different biochemical test. The highest prevalent bacteria was found to be *Staphylococcus aureus* 79 (42.25%) followed by *Streptococcus agalactiae* 27 (14.43%), *Escherichia coli* 25(13.38%), Coagulase Negative *Staphylococcus* species 24 (12.83%), *Enterococcus faecalis* 21 (11.23%) and *Streptococcus dysgalactiae* (5.88%) in that order (Table 3).

## DISCUSSION

Bovine mastitis remains a serious and common disease in animals with significant economic losses in dairy industry worldwide [15]. Currently, in Ethiopia especially in the central highlands where most of the dairy farms are found, the information on prevalence, its associated risk factors and major mastitis causing bacteria species is scarce. Therefore, the objectives of the current study was to determine the prevalence, its major causative bacteria species and risk factors associated with the occurrence of bovine subclinical mastitis.

The overall prevalence of bovine subclinical mastitis was 70.62 % at cow level in this study area. The present findings was comparable to the findings of [9,16-18], who got 69.8% in dairy farms of Addis Ababa and its vicinity, 71% in dairy farms of Holeta town , 71.1% from Holeta and 74.7% around Addis Ababa, respectively. The finding was higher than previous reports of [19-22] who reported 40.4% in

**Table 2: Prevalence of bovine mastitis at cow level in different districts in central highland Ethiopia.**

Districts	No. of examined cows	No. of positive cows	Prevalence (%)
Farm A	154	91	59.09
Farm B	13	11	84.61
Farm C	27	27	100
Farm D	79	57	72.15
Farm E	30	28	93.33

**Table 3: The frequency of bacteria isolated from bovine mastitis in selected districts of central highland of Ethiopia.**

Bacterial species	Total number of isolates	Prevalence (%)
<i>Staphylococcus aureus</i>	79	42.25
CNS*	24	12.83
<i>Streptococcus agalactiae</i>	27	14.43
<i>Streptococcus dysgalactiae</i>	11	5.88
<i>Enterococcus faecalis</i>	21	11.23
<i>Escherichia coli</i>	25	13.38
Total	187	100

\*CNS = Coagulase Negative *Staphylococcus* species

southern Ethiopia, 38.2% in Adami-Tulu central Ethiopia, 39.8% in and around Addis Ababa, and 39.7% in Chaffa valley in north eastern Ethiopia, respectively. However, it is lower than the reports of [23] who indicated that 81.0% were positive for mastitis at cows' level in Holeta, central highland of Ethiopia, The variation in prevalence and incidence of mastitis between the different studies might be partly due to different types of diagnostic tests, sampling procedures and associated risk factors such as age, number of parity, visible teat abnormalities, husbandry practice, barn floor status, milking hygiene, and breed of the animals included in the studies.

Among the risk factors considered; age, number of parity, visible teat abnormalities, husbandry practice, barn floor status and milking hygiene were found to be statistically significant ( $p < 0.05$ ) with the occurrence of mastitis. The result showed that the prevalence of mastitis was significantly higher (81.97%) in cows older than 10 years followed by cows in the age range of 6 to 10 years and lowest in cows younger than 6 years. There was statistically significant difference





among different age groups. This finding is in broad agreement with reports made by different authors in different parts of the country [24]. Who reported age considered as potential risk factor to mastitis and older cows were more affected by mastitis than younger cows, this finding is also agreed with previous reports on mastitis in Southern Ethiopia in holeta area and it's surrounding [17]. The increase in prevalence rate with the advancing age may be due to gradual suppression of immune system of the body, structural changes in udder and teats and repeated exposure to milking practices.

The highest prevalence of subclinical mastitis was observed (90.00%) in cows with parity of more than 6, followed by less than 3 and 3-6 parity. The highest prevalence of mastitis was observed (91.66%) in cows with two teat blind, followed by teat laceration/lesion (71.42%) and one teat blind (67.39%). This is in agreement with [21,25,26] who identified parity as risk factor to mastitis in the study conducted at different parts of Ethiopia. This might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis control program and associate with the ability of the immune system of an animal to defend infection causing agents.

Intensively managed cows present a higher risk for the development of mastitis (84.04%), followed by semi-intensive (64.59%), with least risk among extensively managed animals. This result was line with previous reports on mastitis in [27]. Housing increases the risk of mastitis because of the confinement of the animals, and the multiplication of pathogens in the litters elevates teat challenge, and consequently mastitis. Mastitis prevalence increases in herds housed under poor stable and drainage conditions. This is much more evident for coliform mastitis [28]. Several factors in the environment affect the exposure of a cow to microorganisms. Sources of environmental exposure are manure, bedding, feeds, dirt, mud and water. A good example of this is *Escherichia coli*, which is present in the environment of the cow. Several studies have indeed linked the cleanliness of the barn, and the colony count in the bedding with the incidence of clinical mastitis [29]. Prevalence of mastitis was significantly ( $p < 0.05$ ) associated with milking hygienic practice. Cows at farms with poor milking hygiene standard (96.07%) are severely affected than those with good milking hygiene practices (8.08%). This result agreed with the report of [27,30]. This might be due to high stocking density, dirty bedding or ground, infected utensils, poor ventilation and high humidity.

The result obtained from bacteriological analysis of the samples revealed that from the total of 214 strong CMT positive samples, 187 (87.38%) were culture positive which is higher than who reported proportions of 18% [31]. In this study the predominant organisms isolated from mastitis to be *Staphylococcus aureus* followed by *Streptococcus agalactiae*. The predominance and primary role of *S. aureus* isolate in bovine mastitis has also been reported in other studies [14,17,31]. Different researches finding indicate that, *Staphylococcus aureus* was the most frequently isolated bacteria as per the reports of [16,32] in dairy farms of Holeta and Selalle towns, respectively.

The preponderance of contagious mastitis in this study may be ascribed to the lack of proper milking procedure before milking, during the time of milking and post milking [14]. For instance absence of pre and post teat dipping using antiseptics, washing of milker's hands and using teats secretion as a lubricant of teats at the time of milking which is often practiced in the study area might contributed to the spread of these pathogens from infected teats

to healthy ones. In the present study indicated that environmental bacteria like *Escherichia coli* was isolated in high proportion (9.4%). This is in congruent with the reports of [32,33] who found 7.5% of the total isolates. In contrast, this figure is higher than isolates reported by [16,27] who reported 4.57% and 0.75% in different parts of Ethiopia, respectively. The presence of environmental bacteria might be an implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area.

## CONCLUSION AND RECOMMENDATIONS

The current study showed that an overall prevalence of 214 (70.62%) from total of 303 cows examined bovine mastitis was recorded in the study area. This indicates that mastitis is a serious problem across herds in this study areas. The present study revealed that mastitis has great economic significance associated with reduced production and productivity of cows suffering from mastitis in study area. Among the risk factors considered, age, number of parity, visible teat abnormalities, husbandry practice, barn floor status and milking hygiene were found to be statistically significant ( $p < 0.05$ ) with the occurrence of mastitis. In this study the predominant organisms isolated from mastitis to be *Staphylococcus aureus* followed by *Streptococcus agalactiae*. So, it is a problem for individual particular and the country in general. In line with above facts the following recommendations are forwarded: Awareness creation should be given to the dairy herds on the impacts of bovine mastitis and its associated risk factor, all quarters of the udder of each cow should be periodically checked for the timely treatment and prevention, good record keeping practice on the general herd health of dairy farms, adequate housing with proper sanitation and ventilation should be regularly maintained, Since the bacteria isolated from cows' milk samples in the present study are types that cause both contagious and environmental mastitis, correct and good milking techniques should be applied.

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